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RESPIRATION IN PLANTS

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RESPIRATION IN PLANTS

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WITH TEN DIAGRAMS

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PREFACE

THE supreme importance of respiration, being as it is one of the most universal and fundamental processes of living protoplasm, is recognized by all physiologists. In spite of this, students of botany frequently give respiration little more than a passing consideration.

This curious state of affairs is largely due to the fact that most of the existing accounts of respiration in plants are unsatisfactory because they are either insufficiently comprehensive or insufficiently lucid. In the present book we have aimed at giving an account of the nature of plant respiration which is readable and understandable by the elementary student of botany and which at the same time contains sufficient information to render it of value to the advanced student. We have throughout endeavoured to indicate the principles of plant respiration rather than to catalogue a mass of detailed observations from researches often of very dubious value. Nor have we thought it desirable to enter into a detailed discussion of the very considerable amount of recent work dealing with oxidizing systems in yeast and in animal cells, and the theories based on this work, for the bearing of these observations on the problems of plant respiration is at present not in the least clear.

Reference has, however, been made from time to time to actual researches where we have considered them to be useful as illustrations of various aspects

of the subject. A list of these works is given at the end of the book. No attempt has been made to compile a comprehensive bibliography of books and papers dealing with plant respiration.

W. S.

W. L.

BIRMINGHAM, *1932*

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RESPIRATION IN PLANTS

CHAPTER I

INTRODUCTORY

IN the maintenance of its life, every living thing exhibits a phenomenon which consists essentially in the breaking down of complex substances into simpler ones, with consequent release of energy. This phenomenon has been called ‘ dissimilation ’ in contrast to ‘ assimilation ’ in which simple substances are absorbed and built up into the organism in the form of substances of greater complexity and higher energy content. Although this dissimilation affects different materials in different species, it very commonly involves the breaking down, by oxidation, of carbohydrates and fats, the end-products being carbon dioxide and water. The dissimilation process thus involves an exchange of gases between the organism and its environment, oxygen being absorbed and carbon dioxide evolved. This exchange of gases, so characteristic in animals, is equally characteristic of the vast majority of plants. Hence the term ‘ respiration ’, used to denote this gaseous exchange and the processes of which it forms a part, is equally applicable to animals and plants.

Although the term respiration at first referred to the exchange of gases between the organism and its environment, so that, in the case of animals, it was synonymous with the term breathing, it has for many years now been more usual to regard respiration as involving the whole of the dissimilation

process. Three of the workers to whom the building up of our knowledge of plant respiration has been largely due, Sachs, Pfeffer, and Palladin, have all given the word respiration this wider meaning, and in this book respiration in plants is taken to include all the phenomena of dissimilation, the characteristics of which are the breaking down of complex substances into simpler ones with a consequent release of energy.

Respiration, so defined, is a much more fundamental property of living substance than the exchange of gases between organism and environment, for gaseous exchange is merely an aspect of the most usual form of respiration, and may not always be present, whereas respiration is a property, not merely of every living organism, but of every living cell. It is a constant characteristic of protoplasm. At the same time the cases in which respiration does not involve an exchange of gases are relatively few, and it is no wonder that Sachs, in reviewing the characteristics of plant respiration, laid particular stress in the first case on the importance of a supply of oxygen.

We may, indeed, regard the history of our knowledge of respiration in plants as beginning in the seventeenth century with such observations as that of Malpighi, published in the year 1679, that seeds require a supply of air in order to germinate. It was, however, naturally not until the development of pneumatic chemistry by Priestley, Lavoisier, and others, that the nature of gaseous exchange between organisms and environment could be appreciated. By 1777 Scheele had shown that germinating seeds absorbed and utilized oxygen and produced carbon dioxide, while about the same time Lavoisier began his work on animal respiration which was to put knowledge of that subject on a sound basis. In 1779 Ingen-Housz, in his *Experiments upon Vegetables*, showed that all living plants give out carbon dioxide in the dark, and that non-green plants do so in the light as well.

The serious study of plant physiology began with the introduction of quantitative investigation by de Saussure. In a paper published in 1797 with the title *La formation de l'acide carbonique est-elle essentielle à la végétation ?*, he laid emphasis on the similarity between plants and animals in their production of carbon dioxide, and in their absorption of oxygen from the atmosphere for the formation of carbon dioxide. By actual measurement he was able to show that the volume of oxygen absorbed by germinating seeds was equal to that of the carbon dioxide produced. He dealt at greater length with the subject in his *Recherches chimiques sur la végétation*, published in 1804. In this work he records that with leaves in the dark he found that less carbon dioxide is evolved than oxygen absorbed. He further showed that different leaves respire at very different rates, while he observed the gaseous exchange exhibited by respiring roots, flowers, and fruits.

De Saussure clearly distinguished between the assimilatory gaseous exchange which proceeds in the green parts of plants in the light and the reverse gaseous exchange which proceeds in non-green plants in both light and darkness and in green plants also in the dark. He further showed that germination and growth are dependent on a supply of oxygen.

In a later work, published in 1822, de Saussure showed that the evolution of heat by flowers, which had been observed by Lamarck in *Arum italicum* in 1777, was accompanied by absorption of oxygen, two phenomena which are both features of respiration.

During the next forty years little progress in knowledge of respiration was made. Throughout this period much confusion of thought appears to have resulted through both gaseous exchanges being called 'respiration'. A plant was said to exhibit a diurnal respiration during the day and a nocturnal respiration at night. Nor is it likely that progress in knowledge of these matters was much helped when

such an authority as Liebig denied the existence of a respiration in plants comparable with that of animals. According to him, plants simply absorbed carbon dioxide from the air or soil and later gave it off unchanged when assimilation stopped, much in the same way as water vapour is given off in transpiration.

However, von Mohl, in his *Grundzüge der Anatomie und Physiologie der vegetabilischen Zelle* published in 1851, and also Garreau in the same year, made perfectly clear the difference between these two kinds of gaseous exchange, and definitely indicated the significance of both in the life of the plant. It was not, however, until 1865 that Sachs pointed out what he later called 'the scarcely conceivable thoughtlessness and obtuseness' in 'speaking of a double respiration of plants—of a so-called diurnal respiration, meaning assimilation, and a so-called nocturnal respiration, by which was understood the evolution of carbon dioxide which occurs in true respiration'. From this time onwards the term respiration ceased to be used in connexion with the assimilatory process.

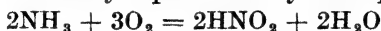
The work and writings of Sachs gave a great impetus to plant physiology, and, from his time, work on respiration has proceeded practically without interruption. Among the numerous contributors to our knowledge of the subject during the last seventy years, perhaps special mention should be made of Pfeffer and Palladin as original workers themselves and as inspirers of many others. During this period research on respiration has chiefly aimed at acquiring information regarding the magnitude of the process and the manner in which it is affected by external and internal conditions. This has been largely with a view to discovering what is generally called the mechanism of the process, such questions being involved as the nature of the materials utilized, the stages in the process, its relation to cell enzymes, the way in which it is linked with other plant processes

and growth, and the part it plays in the life of the plant generally.

At the beginning of this chapter it was pointed out that the essential characteristic of what is now called respiration is not a particular exchange of gases between organism and environment, although this is generally present, but a katabolism or breaking down of more complex substances into simpler ones with a release of energy. In the commonest form of respiration this release of energy is brought about by the oxidation of organic material such as carbohydrates, fats, and proteins, for which a supply of atmospheric oxygen is necessary. This process is known as aerobic or oxygen respiration, and is universal enough to be regarded as the normal mode of respiration in plants. There will obviously be differences in detail according as the material utilized, or *substrate*, is carbohydrate, fat, or some other substance. There are, however, other processes met with in plants which bring about a release of energy. The most important of these is that known as anaerobic respiration in which carbohydrate is broken down to alcohol and carbon dioxide without the participation of atmospheric oxygen, and which is thus similar to, and possibly identical with, the process known as alcoholic fermentation. Probably all plants which normally respire aerobically continue to respire anaerobically, for a time at any rate, when deprived of oxygen. Much work has been done with the object of determining the relationship between these two kinds of respiration.

Anaerobic respiration is normally met with in certain bacteria. Some of these live only in absence of oxygen or in presence of a negligible concentration of this gas. Such organisms are termed *obligate* anaerobes, and include among others such forms as *Bacillus denitrificans* and certain butyric and lactic bacteria. *Facultative* anaerobes, on the other hand, are organisms which normally require oxygen but

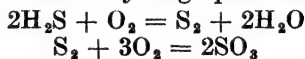
which can live anaerobically when grown on suitable media. Certain butyric and lactic bacteria also fall into this class as well as *Bacillus phosphorescens* and various thermophile bacteria. Among bacteria there also occur oxidations which appear to serve a respiratory function, and in which not an organic substrate, but an inorganic one, is oxidized. The best known of these are the nitrifying bacteria, *Nitrosomonas* and *Nitrococcus*, which obtain energy by oxidising the ammonia of ammonium salts to nitrites. The oxidation is usually represented by the equation :

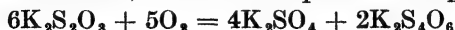
$$2NH_3 + 3O_2 = 2HNO_2 + 2H_2O$$

A second type of oxidation is present in the nitrating bacteria *Nitrobacter* which oxidise nitrites to nitrates :

$$2HNO_2 + O_2 = 2HNO_3$$

Apparently the energy obtained by these respiratory processes is sufficient for the life of these organisms, for there appears to be no respiration of organic material in them. Further, with the energy so obtained they are able to assimilate carbon dioxide without the necessity of absorbing light energy as in green plants.

Similar to the nitrifying and nitrating bacteria are the sulphur bacteria, including *Beggiatoa, Thiothrix,* and *Hillhousia,* which utilize hydrogen sulphide for respiratory purposes. The hydrogen sulphide is oxidised to sulphuric acid, free sulphur being formed in an intermediate stage and appearing in the cell in the form of relatively large particles :

$$2H_2S + O_2 = S_2 + 2H_2O$$
$$S_2 + 3O_2 = 2SO_3$$

A further group of bacteria, the thiosulphate bacteria, *Thiobacillus*, oxidise thiosulphates to sulphates :

$$6K_2S_2O_3 + 5O_2 = 4K_2SO_4 + 2K_2S_4O_6$$

The iron bacteria, *Spirophyllum ferrugineum, Crenothrix polyspora,* and others, are said to utilize ferrous iron for respiration, oxidising it to the ferric condition. It has been suggested that the action is as represented in the following equation :

$$2Fe(HCO_3)_2 + O + H_2O = Fe_2(OH)_6 + 4CO_2$$

Some doubt has, however, been cast on the view that these bacteria utilize ferrous salts in this way.

The hydrogen bacteria, *Hydrogenomonas spp.*, *Bacillus hydrogenes*, and *B. pantotrophus*, oxidise hydrogen to water, and, like the other forms mentioned above, obtain enough energy from this reaction to enable them to assimilate carbon dioxide without a supply of radiant energy.

These various kinds of respiration met with in bacteria are interesting and important in that they help to indicate the meaning of the respiratory process. They are, all the same, limited to a very few organisms, which, although plants, belong to a very highly specialized group. They will not be dealt with further in this book.

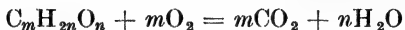
It has been noted that the essential property of respiration, whatever form it may take, is the release of energy. Every living cell respires and therefore presumably requires a supply of energy. This is generally accepted as a fact, but the reasons for it are often stated in the vaguest terms. One considerable worker in this field states that ' vital combustion . . . causes the mysterious apparatus of the living protoplasm to function ', another that the ' energy is used in other processes that go on within the plant '. There are indications that part, perhaps much, of the energy released in respiration is dissipated in the form of heat, and can be measured as such, and that only a small proportion is transformed into mechanical or chemical energy. Some is presumably used in the building up of more complex compounds from inorganic materials and the sugars formed in photosynthesis, and in the building up of protoplasm itself, but it is not known, as Pfeffer pointed out thirty-five years ago, whether or not the respiratory processes involve a continual destruction and re-formation of the protoplasm. Some energy is no doubt utilized in streaming movements of proto-

plasm and other movements of material in th plant. But, apart from the few plant bodies tha have the power of active locomotion, there does nc appear anywhere in the vegetable kingdom a neces sity for a large supply of energy comparable wit that obviously required for the maintenance of th life of a free-moving animal. Nevertheless, we mus suppose that a certain and continuous supply c energy is as necessary for the plant as for the animal for of all the characteristics of living matter, nonc is more constant than the presence of respiration, and nothing is more characteristic of the active living plant cell than the continuous incidence o this process.

It has been stated above that de Saussure initiatec a new era in plant physiology in that he introducec quantitative measurement into his researches. Th advance of knowledge of respiration, as of every plan process, has depended largely on its measurement We have noted that the respiratory process is not constant throughout the plant kingdom and that its essential characteristic, a release of energy, may be effected in different ways. However, in the vast majority of cases respiration consists of a slow oxidation of material, of which the outward signs are a consumption of oxygen and elimination of carbon dioxide. This is the process which, as we have already indicated, is known as aerobic, or oxygen respiration, or sometimes as normal respiration. Here, theoretically, respiration could be studiec quantitatively by determining either the oxyger consumption or the carbon dioxide evolution exhibitec by the respiring tissue, and in practice the determination of one or other of these quantities usually forms the basis of respiration measurement. The loss o material in respiration would also give a measure of the process, but such determinations are no always practicable. The measurement of respiratory activity, therefore, generally resolves itself into either

a determination of oxygen absorption or carbon dioxide evolution.

Where the substrate of respiration is a carbohydrate the complete oxidation of the carbohydrate to carbon dioxide and water involves the consumption of a volume of oxygen equal to that of the carbon dioxide evolved according to the general equation:

$$C_mH_{2n}O_n + mO_2 = mCO_2 + nH_2O$$

When this relation is actually maintained it does not matter whether the respiration is measured by determining oxygen absorption or carbon dioxide evolution. In many cases, however, as will be seen in the next chapter, the volumes of oxygen absorbed and carbon dioxide evolved, owing to a number of reasons, are not the same. For example, a plant which normally respires anaerobically will still give out carbon dioxide in absence of oxygen, and in low concentrations of oxygen the volume of carbon dioxide evolved may exceed that of oxygen absorbed, as if respiration were partly aerobic and partly anaerobic. In such cases, and wherever there is evidence of a change in the ratio of carbon dioxide evolved to oxygen absorbed, as well as in many other instances, a knowledge of both the rate of oxygen absorption and carbon dioxide evolution is desirable.

Various forms of apparatus have been devised for measuring the oxygen absorbed and the carbon dioxide evolved by respiring plants. In the simplest types of apparatus the respiring material is enclosed in a vessel containing a gas mixture of known composition. After a lapse of a suitable time the carbon dioxide is determined by observing the reduction in volume at constant pressure after this gas is absorbed by potassium hydroxide, while the oxygen can be similarly determined by the use of pyrogallol. Where the volume of gas available is sufficient for exact determination, the changes in composition of the gas mixture can be measured by the gas-

analysis apparatus of Haldane. Frequently, however, the quantity of gas available is insufficient for this, and in consequence of the need for determining the carbon dioxide and oxygen changes in relatively small quantities of gas, various so-called micro-eudiometers have been devised, the best known of which is that of Bonnier and Mangin. The principle involved in the use of this apparatus is, however, the same as that of an ordinary gas-analysis method. It is claimed that oxygen can be determined to 0·5 per cent. and carbon dioxide to 0·3 per cent. of the total volume.

A variant of this method consists in absorbing the carbon dioxide evolved in a solution of an indicator. The colour change produced depends upon the amount of carbon dioxide absorbed and can be estimated by matching the indicator with standard tints.

The authors have developed the instrument known as the katharometer for the measurement of carbon dioxide evolution. Here the change in resistance of a spiral of platinum wire consequent on changes in the concentration of carbon dioxide in the gas surrounding the wire forms the basis of the measurement. By taking necessary precautions, carbon dioxide can be determined to 0·001 per cent. of the total volume. This instrument is thus 300 times as sensitive as the apparatus of Bonnier and Mangin and can be used for very small quantities of gas. It has also an advantage over most other methods of measuring respiration in that a continuous record of carbon dioxide evolution can be obtained with it.

It is frequently an advantage in measuring respiration not to keep the respiring tissue in a closed chamber but to pass a continuous current of gas of known composition over the material. The carbon dioxide is then absorbed from the gas after it leaves the respiration vessel. In the most usual form of apparatus the gas bubbles through a tube, the well-

known Pettenkofer tube, containing a standard solution of barium hydroxide, for a definite time, the carbon dioxide absorbed being then determined by titration. Instead of determining the carbon dioxide by titration, Spoehr measured the electrical conductivity of the solution, the fall in electrical conductivity being a measure of the carbon dioxide absorbed. Other workers have absorbed the carbon dioxide in potassium hydroxide solution and determined the quantity of gas absorbed either from the gain in weight or by titration.

The chief disadvantage of using a continuous stream of gas is that the method is not very sensitive, so that it frequently requires a considerable amount of material and a long period of respiration in order to obtain a single measurement. The chief advantages of the method are that the carbon dioxide does not accumulate in the neighbourhood of the respiring tissue and that a series of measurements can be made over a period of time.

However, both these advantages can be introduced into methods involving the use of a closed system if the respiration chamber forms part of a circulatory system involving also a vessel containing the absorber of carbon dioxide and a pump to effect circulation.

The rate of carbon dioxide evolution or of oxygen absorption having been measured, there still remains the question of how these values can be used to express respiratory activity. Usually the carbon dioxide evolved in unit time per unit of dry matter is taken as a measure of respiratory activity. Palladin attempted to calculate respiratory activity in terms of evolution of carbon dioxide per unit of protoplasm, but it is doubtful if he was really able to obtain a value for the amount of protoplasm in different tissues.

We shall have occasion to refer to this question of measurement of respiratory activity in the next chapter.

CHAPTER II

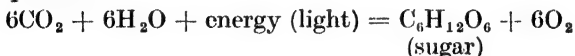
RESPIRATION OF NORMAL PLANTS UNDER AEROBIC CONDITIONS

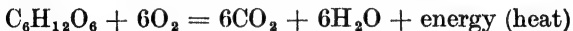
MOST of the common plants with which we are familiar live with the greater part of their external surfaces in contact with the atmosphere. There is therefore available for their use an abundant supply of oxygen. It has already been stated that plants in their normal respiration absorb quantities of oxygen and at the same time give out carbon dioxide. This exchange of gases, which is the outward manifestation of respiration, although continually taking place, may be masked or even reversed in the green parts of plants when they are exposed to light, as a result of photosynthetic activity. During night time, or when a plant is placed in the dark, the absorption of oxygen and evolution of carbon dioxide can always be demonstrated. The exchange of gases takes place over all parts of the plant which are in contact with the atmosphere except where the external walls of the superficial cells are rendered impermeable by impregnation with such substances as cutin and suberin. Within the plant the process is maintained by diffusion between one living cell and another and between the cells and intercellular spaces, the latter usually being in direct communication with the outside air through such channels as stomata and lenticels.

The precise nature of the process which we call respiration is, as yet, little understood. Its existence

and intensity can easily be demonstrated and studied through the medium of the resulting gaseous exchange which is nearly always taking place. We know that it resembles a slow combustion whereby certain complex organic substances are oxidized and broken down into simple substances with an accompanying release of energy. The greater part of this released energy is frequently, if not always, dissipated in the form of heat, and as such can be detected and measured.

The amount of energy that is released when a complex substance is broken down into simple substances is equal to the amount of energy that has to be supplied to the simple substances in order to make them combine and form the complex substance. In the green parts of plants we know that energy from the sun in the form of light is absorbed and used in photosynthesis to bring about the formation of sugar from carbon dioxide and water. This reaction is indicated in a general way by the equation :

$$6CO_2 + 6H_2O + \text{energy (light)} = \underset{\text{(sugar)}}{C_6H_{12}O_6} + 6O_2$$

In the presence of atmospheric oxygen, sugar can readily be made to burn and give out heat, and in the process of burning it is broken down into carbon dioxide and water, as shown in the equation :

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + \text{energy (heat)}$$

The above equation represents the process in its simplest form in which direct combination occurs between gaseous oxygen and sugar. The combination is rapid so that the whole of the energy involved is released as heat in a very short time, which consequently causes a marked rise in temperature in the neighbourhood of the seat of the reaction. In plant respiration we frequently find sugar being broken down to carbon dioxide and water, but the process is more complicated. It involves a chain of reactions

in which a series of intermediate products is formed. Also the quantity of sugar oxidized is relatively small and is distributed through a relatively large mass of tissue. As a consequence, even where practically all the energy is known to be released in the form of heat, the resulting rise in the temperature of the tissue is so small as to be often difficult to measure. This chain of reactions, which is discussed at length in Chapter IV and which results in the breaking down of sugar, is due to the activities of enzymes that are produced within the protoplasm of the living cells where respiration is taking place. It is thus misleading to speak of respiration as combustion. There are, however, two points of resemblance between respiration and combustion; firstly, the substrate and final products may be the same in the two processes, and secondly, the total amount of energy set free will be the same in the two processes provided the end products are the same.

Respiratory Quotient

It will be readily gathered from an examination of the equation on p. 13 that where, in the plant organ, sugar is the substance broken down during respiration under conditions of a plentiful supply of oxygen, with the production of carbon dioxide and water, six molecules of oxygen will be used up for every molecule of sugar respired. As a result of this, six molecules of carbon dioxide are set free. In other words, the ratio of the volume of carbon dioxide evolved to the volume of oxygen absorbed is equal to unity. This ratio is known as the *respiratory quotient.* There is thus an intimate relationship between the value of the respiratory quotient and the composition of the respiratory substrate on the one hand, and the nature of the respiratory process on the other. Where this substrate is carbohydrate the respiratory quotient is almost invariably in the neighbourhood of unity if the respiratory process

results in complete breakdown to water and carbon dioxide. This has been definitely demonstrated by a number of investigators, by measuring the oxygen intake and carbon dioxide output of respiring fungus mycelia growing on culture solutions containing various known substances.

For example, Puriewitsch obtained the values given below for the respiratory quotient in the case of *Aspergillus niger*.

TABLE I

RESPIRATORY QUOTIENTS OF *Aspergillus* ON VARIOUS MEDIA
(*From* Puriewitsch)

10 per cent. Sucrose	10 per cent. Dextrose	10 per cent. Raffinose
1·05	1·17	0·90
1·09	1·19	0·93

Similarly, De Boer obtained values for the respiratory quotient of between 0·99 and 1·21 for *Phycomyces* grown on bread.

In the case of higher plants it is not always easy to correlate respiratory quotient values with the composition of the substrate, as the latter may often be difficult to ascertain. With leaves, however, the problem is fairly simple, as these organs, if removed from the plant during active assimilation, contain abundance of carbohydrates. These carbohydrates, if the leaves are placed in the dark, form the substrate for respiration, and, just as with the fungi mentioned above, the respiratory quotient approaches unity. The table on page 16 gives values of the respiratory quotients in a number of leaves investigated by Maquenne and Demoussy.

It frequently happens in plants that fats and not carbohydrates form the substrate for respiration. Fats require a larger amount of oxygen for their complete oxidation to water and carbon dioxide than is the case with carbohydrates. Thus the complete oxidation of the fat tripalmitin, $C_3H_5O_3(OC.C_{15}H_{31})_3$ involves the utilization of 145

molecules of oxygen for every 102 molecules of carbon dioxide produced:

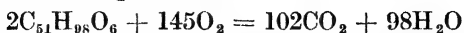
$$2C_{51}H_{98}O_6 + 145O_2 = 102CO_2 + 98H_2O$$

As a consequence, we find that respiration involving the breaking down of fats into carbon dioxide and water results in a respiratory quotient of less than unity. This fact also has been clearly demonstrated by means of the fungus *Phycomyces*. By growing *Phycomyces* on a ground-linseed medium, De Boer obtained values for the respiratory quotient varying between 0·66 and 0·75.

TABLE II

RESPIRATORY QUOTIENTS OF LEAVES

(*From* Maquenne and Demoussy)

Plant	R.Q.	Plant	R.Q.
Ailanthus	1·08	Pea	1·07
Aspidistra	0·97	Pear	1·10
Aucuba	1·11	Poppy	1·09
Begonia	1·11	Privet	1·03
Cherry Laurel	1·03	Rhubarb	1·02
Chrysanthemum	1·02	Ricinus	1·03
Haricot	1·11	Rose	1·02
,,	1·07	Spindle Tree	1·08
Ivy	1·08	Sorrel	1·04
Lilac	1·07	Tobacco	1·03
Lily	1·07	Turnip	1·11
Mahonia (autumn)	0·95	Vine	1·01
Maize	1·07	Wheat	1·03
Oleander	1·05	Wild Grape	1·00

The respiratory quotient has formed a centre of interest in a considerable number of researches on the course of respiration of seeds during germination. As is well known, seeds contain reserve stores of food materials which provide for the needs of the growing plant in its early stages of development. In the majority of seeds this food reserve consists of oil (liquid fat); in some, for example those of the Leguminosae and Gramineae, it may be carbohydrate in the form of starch, or less frequently, hemicellulose or sugar, while in others it appears to be largely protein.

From what has already been said it is clear that the nature of the food reserve in any given seed should affect the value of the respiratory quotient during germination. There is ample experimental evidence to show that this is the case, and in general it may be stated that in the case of seeds with carbohydrate reserve materials the respiratory quotient during the greater part of the germination period approaches unity. On the other hand, with fat-containing seeds, the respiratory quotient falls considerably below unity. The matter, however, is far from being simple, and although a number of published figures showing the changes which the respiratory quotient undergoes during germination of certain seeds are available, the divergences which exist between the results of one investigator and those of another in any given species leave much to be desired. In spite of the lack of reliable information as to the absolute values of the respiratory quotient of germinating seeds, the work of different authors shows some measure of agreement with regard to the way in which it varies as germination proceeds.

Apparently during the first few hours of germination of all the seeds examined, the respiratory quotient is in the neighbourhood of unity or is greater than unity. As germination proceeds the value of the quotient falls. In the case of seeds with carbohydrate food reserve the fall in the value of the quotient may be slight, and in any case continues only for a short time, after which it rises again and approaches unity. With fat-containing seeds the fall in the value of the quotient is very marked and continues usually for a considerable time. Eventually, however, it also rises and approaches unity. This difference in the behaviour of the respiratory quotient for carbohydrate and fat-containing seeds is illustrated by the curves shown in Fig. 1.

It thus appears that the germination of fat-con-

taining seeds can be, broadly speaking, divided into three phases. *Phase* 1: an initial period during which the value of the respiratory quotient is high, or in other words, the output of carbon dioxide is approximately equal to or greater than the intake of oxygen. *Phase* 2: a middle period during which the respiratory quotient decreases to a minimum value owing to a considerable increase in the volume of oxygen absorbed compared with the volume of carbon dioxide given out. *Phase* 3: a final period in which the respiratory quotient rises and may

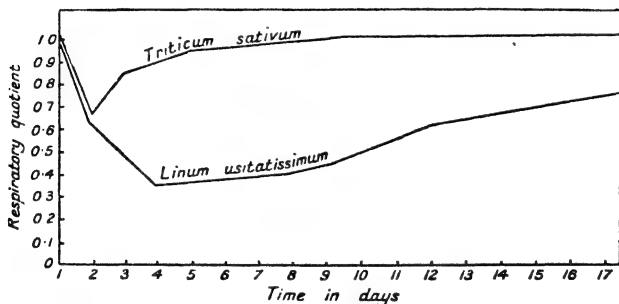



Fig. 1.—Curves showing the changes occurring in the value of the respiratory quotient during the first 17 days of germination and development of seeds of *Triticum sativum* and *Linum usitatissimum*

(*After* Bonnier and Mangin)

approach unity. Theoretical explanations which would appear to account for the differences that exist between these three phases will now be briefly discussed. In the initial phase the respiratory quotient is in the neighbourhood of unity. This is probably due to the fact that when germination is beginning, the respiratory substrate is provided by a small quantity of hexose sugar which is always present in dormant seeds. As water continues to be absorbed by the seed the enzymes which bring about the conversion of fats into carbohydrates are

activated with the result that the respiratory quotient falls. This fall in the quotient which is shown by both typically carbohydrate-storing seeds as well as fat-storing seeds is due to the fact that even in carbohydrate seeds there is present a small amount of fat.

We thus note that phase 2 with its falling quotient is the result of the utilisation of fat for the production of respiratory substrate. In carbohydrate seeds the amount of fat present is usually small and is soon exhausted. The fall in the quotient in their case therefore only continues for a short period at the end of which the respiratory breakdown of carbohydrate again becomes the predominant process and consequently the quotient again rises towards unity. With fat-storing seeds a much longer and greater fall in the quotient occurs as fat continues to be converted into carbohydrate.

In phase 3 an oxidation of carbohydrate substrate, produced as described under phase 2, takes place. This process goes on at the same time as the oxidation of the reserve fats, and its intensity is such that successively determined values of the respiratory quotient follow a rising course.

These theoretical views are further supported by the chemical analyses of germinating hemp seeds published by Detmer and given on page 20.

It will be seen that these three sets of figures roughly correspond with the three germination phases outlined above.

After seven days 15·56 gm. of fat have disappeared, and 8·64 gm. of starch have been formed, which corresponds closely with the respiratory behaviour during the second phase.

After ten days, it will be seen that the amount of fat has been still further reduced and at the same time some of the starch formed in the second phase has disappeared. Some of this starch has presumably been oxidized to water and carbon dioxide while

some has probably been used in the formation of cellulose and protein, both of which substances have increased in quantity.

TABLE III

CHEMICAL CHANGES IN HEMP SEEDS DURING GERMINATION
(*From* Detmer)

	Fat	Starch	Protein	Undetermined compounds	Cellulose	Ash
100 gm.	gm.	gm.	gm.	gm.	gm.	gm.
Ungerminated seeds . .	32·65	—	25·06	21·28	16·51	4·5
After germinating 7 days	17·09	8·64	23·99	26·13	16·54	4·5
After germinating 10 days	15·20	4·59	24·50	26·95	18·29	4·5

When we come to consider the utilization of proteins by plants as respiratory substrates, we are faced with a serious lack of knowledge. Apparently certain information has resulted from experiments with fungi growing on protein culture media. It has been stated that fungi only consume proteins when there are no available supplies of carbohydrate or fat; also that the respiration of proteins results in a low respiratory quotient with a value in the neighbourhood of 0·50. In regard to the higher plants, Bonnier and Mangin, working on the germination of lupin seeds, obtained an average value of 0·54 for the respiratory quotient. This may be due to the fact that lupin seeds contain an abundance of protein serving in the capacity of an auxiliary food reserve, although carbohydrate is present in the form of abnormally thick cell-walls. It seems probable that we have a further instance of protein respiration in the case of leaves that have been kept in darkness for prolonged periods. In such cases it has been found that the respiration rate gradually

falls until it reaches a minimum constant level which is maintained for a considerable time if the leaves remain alive. This minimum constant respiration rate of starved leaves has been termed by Blackman 'protoplasmic respiration' in contradistinction to 'floating respiration', in which carbohydrates or other reserve materials are utilized. The protoplasmic respiration may be the result of the utilization of protein as a respiratory substrate after all reserve materials have been used.

With regard to the cases we have just been discussing, the value of the respiratory quotient is chiefly considered from a relatively simple standpoint, namely, where substrates of highly complex chemical composition are completely broken down to simple compounds such as water and carbon dioxide. It may be assumed with a reasonable degree of certainty that such katabolic processes are useful to the plant mainly in so far as they set free energy. As has already been shown, the value of the respiratory quotient here depends upon the composition of the original substrate. There is, however, another aspect of the question to be considered. In the vast majority of plants, sugar may be taken as the fundamental substance from which by additive or subtractive processes the whole physical and physiological fabric of the organism is constructed. So far as we know, respiration is the universal accompaniment of life, and consequently, in the living plant, sugar is continually being utilized for the formation of other less complex or more complex substances. In the formation of these substances the sugar may be able to supply the exact amount of oxygen required, or on the other hand, additional oxygen may be needed, or surplus oxygen may be set free. In other words, the value of the respiratory quotient will depend upon the substance or substances that are being formed. A considerable amount of experimental evidence con-

firming this point is available, some of which will now be dealt with.

One of the first of such cases that suggests itself to us is that of the maturation of seeds which have fatty food reserves. We have seen that in the germination of such seeds, the breaking down of the fats first to sugars and later to carbon dioxide and water, results in a respiratory quotient of a value less than unity. As these fats, during maturation of the seed, are formed from sugars, one would expect, during maturation, the respiratory quotient to be greater than unity since the change from sugar to fat involves an elimination of oxygen. This has been experimentally demonstrated, as will be seen from the figures obtained by Gerber for linseed. During the maturation period immediately before ripening he found the average value of the respiratory quotient for six seeds to be 1·22, while during the germination of these same seeds the respiratory quotient fell to the average value of 0·64.

Then we have the often-cited case of succulent plants, in the cells of which accumulations of organic acids occur; malic acid in the Cactaceae and Crassulaceae, and oxalic acid in species of *Mesembryanthemum*. When these plants are placed in darkness, the amount of oxygen absorbed in the process of respiration is in excess of the amount of carbon dioxide evolved; in some extreme cases oxygen may be absorbed in marked quantities while no carbon dioxide is given off. Aubert, working with a species of *Opuntia* placed in darkness, obtained a mean value of 0·03 for the respiratory quotient. After the plants have been kept in darkness for a time, the accumulation of organic acids slows down and the rate of evolution of carbon dioxide gradually increases, with a corresponding increase in the value of the respiratory quotient, which, however, does not reach unity. A similar rise in the value of the quotient is produced by an increase in temperature.

When the plants are exposed to sunlight, the acids are decomposed and carbon dioxide is set free. Thus there occurs an accumulation at night of organic acids, which, in the morning, are broken down with liberation of carbon dioxide. This latter gas is available for use in the process of assimilation. It has been suggested that this peculiar metabolism is beneficial in the case of plants like succulents, in which, owing to the massive construction of their assimilatory organs, interchange of gases with the atmosphere may be relatively slow.

A somewhat similar case to that of succulents is that of plants whose leaves are coloured red by the presence of anthocyanin in their cells. Nicolas compared the respiration of green leaves with that of red leaves either from the same plants or from varieties of the same species whose leaves are red. He found that in every case examined the respiratory quotient had a lower value in the case of red leaves than in the case of green leaves. He also found that these differences in the quotients were in every case due to a more active absorption of oxygen by the red leaves than by the green leaves. These differences in the quotients can apparently be related to a greater accumulation of organic acids in the leaves containing anthocyanin than in those from which this pigment is absent. The figures in Table IV, taken from Nicolas, show the amount of acetic acid (milligrammes per gramme fresh weight) in green and red leaves of four species examined, together with the respiratory quotients obtained.

Certain external physical conditions have been found to influence the value of the respiratory quotient. Temperature may markedly affect it in so far as it determines the velocity of the oxidation processes. In the already mentioned case of succulents, where increase in temperature results in the decomposition of organic acids, a marked increase in the value of the respiratory quotient is

brought about. An increase in the value of the respiratory quotient with increase in temperature is recorded by Harrington in the case of apple-seeds. An interesting fact which is probably connected with this, is that sugars and organic acids have been found by a number of workers to accumulate in dormant structures when they are stored at low temperatures.

TABLE IV

RESPIRATORY QUOTIENTS AND ACID CONTENT OF GREEN AND RED LEAVES

(*From* Nicolas)

	Green leaves		Red leaves	
	Mgs. Acetic acid.	$\frac{CO_2}{O_2}$	Mgs. Acetic acid.	$\frac{CO_2}{O_2}$
Raphiolepis ovata	2·88	1·01	6·48	0·81
Photinia glabra	5·85	0·90	6·66	0·77
Acokanthera spectabilis .	8·21	0·94	11·11	0·71
Prunus cerasifera	6·60	0·80	—	—
Prunus cerasifera var. *Pissardi*	—	—	10·80	0·70

If the concentration of oxygen in the atmosphere surrounding the respiring tissue is reduced below a given value, which varies with the plant material used, a marked rise in the value of the respiratory quotient results. This fact was clearly brought out by the researches of Stich in 1891. He found that the percentage of oxygen in the experimental atmosphere could be reduced from that of normal air, namely 20·8 per cent., down to values in the neighbourhood of 5 per cent. without bringing about any marked alteration in the respiratory quotient. When this lower limit of oxygen concentration, the exact value of which depended upon the species of the respiring plant, was passed, a sudden increase in the quotient occurred. This is shown by the figures

given in Table V, which are taken from Stich's paper.

TABLE V

EFFECT OF OXYGEN CONCENTRATION ON RESPIRATORY QUOTIENT

(*From* Stich)

Experimental material	Percentage of oxygen in atmosphere	$\frac{CO_2}{O_2}$
Triticum vulgare, seedlings .	20·8	0·98
	9·0	0·94
	5·0	0·93
	3·0	3·34
Zea Mais, seedlings	20·8	0·89
	9·0	0·96
	5·0	1·35
	3·6	1·37
Pisum sativum, seedlings . .	20·8	0·83
	9·3	0·86
	3·5	2·31
Narcissus poeticus, bulb . .	20·8	0·96
	10·2	1·04
	7·5	2·36

Increases in the concentration of carbon dioxide in the atmosphere surrounding the plant have a very marked depressing effect on the intensity of the respiratory processes, as will be seen later (p. 45). They also bring about a lowering of the respiratory quotient by causing a greater depression in carbon dioxide output than in oxygen intake. (See Table X, p. 46.)

From the foregoing it thus appears that a study of the respiratory quotient may afford interesting clues as to the nature of the respiratory processes that are taking place within the plant. It has

already been pointed out that, as many reactions may be safely assumed to be taking place in plant cells at one and the same time, it may be somewhat misleading to consider respiration as a simple physiological combustion involving the breaking down of a substrate to carbon dioxide and water with the absorption of oxygen. It is possible that more than one substrate may often, if not invariably, be involved; also it is possible that a number of reaction chains may simultaneously exist, giving rise to a diversity of final products. Each of these reaction chains will have its own particular value for the carbon dioxide-oxygen ratio, so that the respiratory quotient for a particular experimental subject, as measured by the experimental means at our disposal, will be the mean of all these reaction chain ratios. It may, as in the case of values obtained from germinating fatty seeds, strongly indicate the nature of the predominant reaction chain that is taking place within the cells of the experimental tissue at the time of the experiment. On the other hand, where no one reaction chain is of sufficient intensity, or has a sufficiently characteristic carbon dioxide-oxygen ratio, to impress itself in an unmistakable way upon the observed respiratory quotient, the value of that quotient will convey little useful information as to what is happening inside the cells of the experimental material. Indeed, it may lead to entirely wrong conclusions; for example, the mean observed quotient resulting from a variety of reaction chains may have a result approaching unity and may consequently convey the idea that a simple complete combustion of carbohydrate substrate to carbon dioxide and water is the predominant chain. The value that is to be placed on such conclusions is obvious, even though it be supported on the part of the experimenter by the usual chemical arguments. These points are mentioned, not with a view to depreciating the existing results

of various investigators, but by way of emphasizing the difficulties that confront workers in this field of research.

Intensity of Respiration

An examination of the various published general accounts of plant respiration reveals the fact that a considerable amount of vagueness exists where attempts are made to deal with the question of the intensity of respiration. In some cases modes of expression are used which are decidedly misleading; for example, one modern author of some repute refers to respiration intensity as respiratory energy, and states that the amount of carbon dioxide given off from a unit of living substance serves as a measure of this. Such a statement, besides being incorrect, involves a misuse of the term energy. Further, the complexity of the process of respiration and the intimacy of its relationship with all the vital processes of the living organism, do not always appear to be taken into account by writers when dealing with respiration intensity. This fact is evident when we consider the criteria most generally used for the purpose of expressing respiration intensity.

As pointed out in Chapter I, these criteria are naturally based on gaseous exchange. Usually the amount of carbon dioxide evolved by the respiring tissue is measured, although occasionally the amount of oxygen absorbed is recorded. These quantities of gas are used as an indication of respiratory intensity by referring them to such quantities as the dry weight of the tissue used, the fresh weight of the tissue used, or the amount of nuclein nitrogen contained in the cells of the tissue.

When, however, we consider the already outlined variations that occur in the value of the respiratory quotient it is clear that estimation of respiration intensity based on the amount of oxygen absorbed, or on the amount of carbon dioxide evolved, may

possess serious inaccuracies. It is true that such criteria, though far from being entirely satisfactory, are useful in an approximate and general way for comparative purposes. We are, however, faced with the fact that no really accurate method of expressing respiration intensity has, up to the present, been devised. This unfortunate state of affairs is due to the fact that in spite of the very considerable amount of research that has been carried out on respiration, we still possess practically no definite knowledge regarding the details of the process. We know that the process begins with some substrate and finishes with certain final products, and that in the process oxygen may be taken in and carbon dioxide given out. The actual respiration intensity, however, depends upon the rate at which the substrate is broken down by respiratory processes and upon the final products. In other words, the true measure of respiration intensity is the rate at which energy is set free, and this rate may or may not be accurately indicated by the rate at which oxygen is absorbed or carbon dioxide liberated. An experimental method, then, is required that will reveal the rate at which energy is set free as the respiratory substrate disappears, but we do not know with certainty what the substrate is. We know a little about it; for instance, we are reasonably certain that it is, in part at least, frequently a sugar or mixture of sugars. We know that other substances such as fats are frequently changed into sugars and the sugars broken down into simpler substances in the process of respiration. Here again, are we, strictly speaking, correct in considering the fat as the respiratory substrate, or should we consider the sugar as such? Moreover, there are vague suggestions that proteins may act as substrates, though information on this point is very incomplete and unsatisfactory.

These facts should be kept in mind when studying the data at our disposal relating to respiration

intensity and the factors which influence it. We will now proceed to a consideration of these data.

It is to be expected that as the various members of the vegetable kingdom differ so widely from each other morphologically, they also differ just as widely physiologically. Accordingly we find great differences between the respiration rates of different species. Also, as no two individuals of the same species are exactly similar morphologically, so no two such individuals exhibit exactly the same respiration intensity when subjected to similar external conditions. Amongst the most actively respiring plants are the fungi and bacteria. For example, Kostychev, working with a two-day-old culture of *Aspergillus niger* on quinic acid at 16° C., found that it gave out 78·08 cubic centimetres of carbon dioxide per gramme of dry weight. Vignol found that a culture of *Bacillus mesentericus vulgatus* at 16° C. absorbed 48·51 cubic centimetres of oxygen per gramme dry weight per hour. Some indication of the type of variation exhibited by the respiration intensity of different species of flowering plants is shown in the following table of values obtained by Aubert.

TABLE VI

RESPIRATION INTENSITY OF VARIOUS PLANTS

(*From* Aubert)

Plant	Temperature in ° C.	Vol. of oxygen absorbed per gramme fresh weight per hour
		c.m.
Cereus macrogonus . . .	12	3·00
Mamillaria cliphatidens . .	12	5·60
Sedum dendroideum . . .	12	19·00
Mesembryanthemum deltoides	12	57·8
Lupinus albus	12	73·7
Vicia faba	12	96·6
Triticum sativum	13	291·00

In general, shade plants and succulent plants respire less actively than more normal types.

Then again we find that different parts of the same plant respire at different rates. In the higher plants, actively growing regions such as meristems and their adjacent immature tissues respire more actively than tissues which have reached their full development. Reproductive structures such as flowers show respiration intensities above the normal average intensity for the whole plant, and in the flower itself, the gynoecium and androecium respire more actively than the sepals and petals. A considerable amount of experimental data has been published by various workers proving these points, a few examples of which will now be considered.

Kidd, West, and Briggs measured the respiration of sunflower plants throughout their development from germination to maturity, and they also obtained comparative data of the respiration intensity of the different organs of mature plants. Their respiration intensities were expressed as milligrammes of carbon dioxide per gramme of dry weight of respiring cell-matter per hour at the temperature of 10° C., the external concentration of oxygen being that of the atmosphere. The respiration intensity so expressed they termed the *respiratory index*.

In Table VIII (p. 33) are values taken from Kidd, West, and Briggs showing the respiratory indices of the various organs of sunflower plants of different ages.

Comparative data showing the relative respiration intensities of leaves and floral organs in the case of four flowering-plant species are given in the following table from results published by Maige in 1911.

Variations in Respiration Intensity during Development

The variations in respiration intensity during the germination of seeds and during the early stages of the development of seedlings have received a con-

siderable amount of attention from experimenters. De Saussure and a number of other pioneer workers recorded the fact that respiration, as measured by carbon dioxide output during the early stages of germination of seeds, showed a gradual increase with development. This work was carried further by Mayer in 1875 and Rischavi in 1876. These two workers observed the respiration of germinating wheat, the former measuring oxygen uptake and the latter carbon dioxide output. They both found that the respiration intensity increased from a very low value up to a maximum, and then gradually fell off in intensity.

TABLE VII

RESPIRATION INTENSITY OF VARIOUS PLANT ORGANS

(*From* Maige)

Species	Temp. in ° C.	Respiration Intensity (c.c. CO_2 per gramme fresh weight per hour)				
		Sepals	Petals	Stamens	Pistil	Leaves
Verbascum thapsus . .	23·0	0·747	0·177	0·761	0·815	0·382
Penstemon gentianoides .	23·5	0·571	0·398	0·602	0·689	0·300
Papaver rhoeas	22·0	0·390	0·367	1·041	0·690	0·332
Lavatera olbia .	22·0	0·615	0·303	0·576	0·894	0·394

More recently, in 1923, Fernandes, using modern experimental methods, examined the respiration rate of germinating peas, and the results of two of his experiments, conducted at 20° C. and 25° C. respectively, are shown in the curves given in Fig. 2.

It will be seen from this figure that Fernandes' results for *Pisum* agree with those of Mayer and Rischavi, the respiration intensity increasing fairly rapidly to a maximum value and then gradually decreasing. It has been suggested by Fernandes that the decrease after the maximum intensity has

been reached is possibly due to exhaustion of available supplies of mineral salts.

The time during which this increase of respiration intensity up to a maximum value is taking place has been termed the grand period of respiration owing to the resemblance which exists between it and the grand period of growth. It is likely that respiration intensity largely runs parallel with growth-rate, but the two values are not always influenced in the same way by similar external

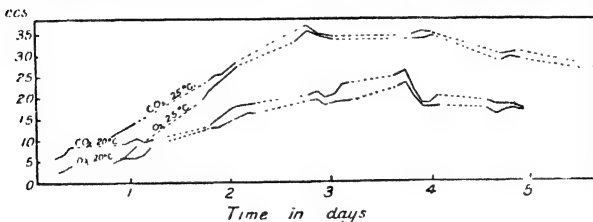



Fig. 2.—Curves showing changes in the respiration of *Pisum sativum* as measured by oxygen intake and carbon-dioxide output during the first five days of germination at temperatures of 20° C. and 25° C., respectively. In the upper curve respiration intensity is indicated as c.cs. CO_2 or O_2 per 50 seeds per 3 hours, in the lower curve as c.cs. CO_2 or O_2 per 50 seeds per 2 hours

(*After* Fernandes)

conditions. For example, it has been shown in the case of germinating wheat that whereas the respiration intensity is higher at a temperature of 34° C. than at 23° C., the growth-rate is lower.

When considering the respiration of more mature plant organs, we find that the intensity of the process tends to decrease with the age of the organ in question. This point has been clearly brought out by Kidd, West, and Briggs in the case of the sunflower. Table VIII summarizes their work in this connexion and shows how the respiratory index (see p. 30) decreases with age in the case of the various individual parts of the plant.

TABLE VIII

Changes in Respiratory Index of Sunflower Plants with Age
(*From* Kidd, West, and Briggs)

Days from germination	No. of plants used	Dry weight of a single plant	Respiratory index (mgm. CO_2 per gm. dry weight per hour) of—					
			Entire plant	Stem	Leaves	Stem apex	Total inflor-escences	Flowers on lateral shoots
1	30	0·0225	2·90	—	—	—	—	—
2	25	0·0223	3·00	—	—	—	—	—
4	25	0·0242	3·00	—	—	—	—	—
13	10	0·1009	2·80	—	—	—	—	—
22	8	0·630	3·00	(3·00)	(3·00)	3·00	—	—
29	2	4·065	2·30	—	—	—	—	—
36	1	12·85	1·21	0·81	1·56	—	—	—
43	1	22·05	1·03	0·69	1·38	—	—	—
50	1	45·15	0·94	0·46	1·52	2·56	—	—
59	1	93·20	0·66	0·33	1·32	1·78	—	—
64	1	98·30	0·71	0·34	1·24	—	—	—
89	1	294·7	0·48	0·31	0·90	1·13*	1·13	—
99	1	377·4	0·37	0·25	0·45	0·89	1·04	1·13
112	1	818·3	0·26	0·098	0·375	0·75	0·85	0·95
136	1	419·5	0·39	0·081	0·44	0·96	0·965	0·97

* From this date onwards the stem apex was the inflorescence only.

Other investigators working with flowers, stems and leaves of various plants, generally speaking, record a similar decrease in respiration intensity with age.

As a result of the most recent work dealing with the relation between respiration rate and the age of plant organs, a further point has revealed itself. In the case of some leaves and fruits examined, the already mentioned decrease in respiration intensity with age continues apparently until the structure is fully developed. The organ then passes into a senescent phase of its existence, during which the respiration intensity increases up to a maximum value, after which it again decreases.

The most complete work on this point is that carried out by Blackman and Parija in 1928 and by Kidd and West in 1930 on the respiration of stored apples. The course of the respiration intensity of apples during storage is shown in Fig. 3, taken from the paper by Kidd and West. In the three curves shown in this figure which indicate the respiratory activity of stored Bramley's Seedling apples at different temperatures, the respiration intensity, in each case, is seen to increase to a maximum value and then to decrease. Similar results have been obtained by Gustafson for the tomato, and by Olney for the banana.

A tentative theory is put forward by Blackman and Parija as a possible explanation of this behaviour of respiration of stored fruits. The essence of this theory, to which further reference will be made, is that during the senescent phase the protoplasmic control of hydrolysis, which they term 'organization resistance', is weakened, with the result that hydrolysis increases and produces an increased amount of substrate for respiration. This increased amount of substrate results in a rise in the respiration intensity. Later, the starvation factor comes into operation and the supply of substrate available for hydrolysis

diminishes, with the result that the respiration intensity decreases, thus causing the later downward slope of the curve from the maximum shown in Fig. 3.

Two other factors that may be termed internal, and which influence the intensity of the respiration process in plants, are water content and seasonal periodicity. Various workers have investigated the effect on respiration of changing the water content

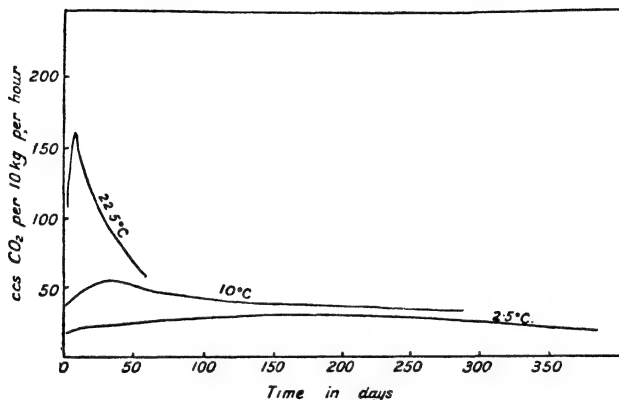



Fig. 3.—Curves showing the course of respiration intensity of Bramley's Seedling apples during storage at temperatures of 22·5° C., 10° C., and 2·5° C.

(*After* Kidd and West)

of the cells of vegetative organs of plants. In some cases this change in water content was brought about by simple desiccation, in others by the osmotic action caused by immersion of the tissue in distilled water or solutions of glucose of different concentrations. In the case of the majority of plants experimented upon in this way, it has been found that, up to a point, increase or decrease in water content brings about an increase in respiration intensity, increase in water content causing greater increase in

respiration than decrease in water content. Amongst the exceptions to this rule are asparagus and paeony which show no increase in respiration rate when their water content is reduced. In every case, as one would expect, desiccation, if carried beyond a certain limit which varies with different species, brings about a reduction in respiration intensity which continues to zero as complete desiccation and death of the tissues is reached. In the case of potato tubers it has been found that withdrawal of water results in a decrease in respiration rate ; a probable parallel to this is the decrease in respiration intensity exhibited by seeds during the process of ripening.

A somewhat similar case in which plant tissues lose water and pass into a resting stage is that of mosses and lichens. Many of these plants are able to withstand extreme desiccation while still remaining alive, and are able, when opportunity favours, to re-absorb water and resume their vital activities. A number of workers have investigated the relationship between water content and respiration rate in the case of various moss species, and have shown that the two values always vary in the same direction. By way of example, the following set of figures obtained by Jönsson for *Mnium undulatum* may be taken.

TABLE IX

EFFECT OF WATER CONTENT ON RESPIRATION INTENSITY OF *Mnium undulatum*

(*From* Jönsson)

Percentage water content	Carbon dioxide evolved per gramme dry weight per 10 hours c.c.
40	0·750
59	1·350
65	3·900
84	9·680

The increase in respiration intensity that is observed in seeds during the period in which they

are absorbing water preparatory to germination is a further case of a similar nature to those discussed above.

The effect of seasonal periodicity on the intensity of respiration presents certain features of interest. Bonnier and Mangin in 1885 found that in the case of perennial plants growing in temperate climates the average respiration was greatest in spring, and showed a slight decrease in summer ; a more rapid decrease to a minimum value occurred with the onset of winter, after which the intensity again increased with the return of spring. In the course of this yearly cycle, two subsidiary maxima were observed, the first being related to the expansion of new leaves in the spring, and the second appearing at the time of flower production.

Attention has more recently been drawn to the question of seasonal effect by the experiments of Inamdar and Singh carried out at Benares on the respiration of the leaves of *Artocarpus integrifolia.* It will be noted here that the plants are growing under tropical climatic conditions. In this region spring occurs at the end of March, during which period the plants produce new leaves and shoots. Summer quickly follows spring and is very hot and dry until the end of June, when monsoon rains begin and continue until the end of September. After the rainy season comes a comparatively dry autumn of about two months' duration, followed by winter, with a relatively low temperature and only very occasional rain.

The general course of respiration of the leaves of *Artocarpus* during the year was found to be as follows: at the beginning of summer, respiration intensity falls to a minimum value which persists until the coming of autumn, it then gradually increases to a maximum level which continues through the winter and spring, again falling as spring gives place to summer.

A striking difference was found to exist between the respiratory behaviour of leaves collected during the summer and those collected during the winter. When leaves were kept in darkness, and their respiration rates measured over periods of several days, leaves collected in winter gave the typical starvation curve in which respiration intensity first rapidly decreased as carbohydrate reserves were depleted until a steady low respiration level was reached and maintained, that is, the change from the 'floating' to the 'protoplasmic' types of respiration of Blackman was observed. In the case of leaves collected in summer and similarly treated, the initial phase was found to be absent, and instead a low, almost uniform, respiration rate was maintained throughout the experiment.

It would appear that the difference between the respiratory intensities of the two seasons might be related in some measure to the relative abundance of respirable carbohydrate substrate, as photosynthetic activity is also at a minimum during summer. More complex causes, however, underlie the matter, as is pointed out by the authors of the work. Some factors apparently bring about a depression of the activity of the metabolic mechanism of the cells of the plant during the summer, this depression affecting both respiration and assimilation alike.

Water content cannot be the determining factor as this is practically the same in summer as in winter. Growth activity also bears no relationship to respiratory activity as the former is practically at a minimum while the latter is at a maximum, that is, during autumn and winter; active growth takes place in early spring and during the rains of late summer. As regards temperature, we find a contrast between plants of temperate regions and those of Benares, in that in the case of the former, the minimum respiration intensity is related to the lowest temperature.

The Effect of External Factors on Respiration Intensity

The various factors that we have so far considered, that influence the intensity of the respiratory processes of plants, are more or less entirely dependent upon conditions within, or upon the specific nature of the living protoplasmic system of the cells. In addition to these are a number of environmental factors that are found to influence plant cells directly or indirectly in such a way as to bring about changes in their respiration rates. These factors may be generally termed external factors, the chief of them being temperature, light, changes in the composition and pressure of the external atmosphere, and the introduction of various chemical compounds into the respiring cells. The investigation of a number of these factors has received a considerable amount of attention, such work being materially helped by the fact that they can be accurately controlled.

Temperature.—Attempts have been repeatedly made to analyse the effects of temperature changes on the respiratory processes. In some cases investigators have gone to the extent of formulating mathematical laws, which are based on experimental data of varying reliability, connecting temperature with respiration intensity. In the present state of our knowledge such laws cannot be treated with any confidence as regards their validity, and their discussion here would be of little help. In fact, the only generalization that we can so far make with any measure of certainty regarding the point in question is that within certain limits, increase in temperature results in increase in respiration rate.

The investigations dealing with the effect of temperature changes on respiration intensity, generally speaking, fall into two categories, namely, those dealing with rapidly developing structures and actively functioning organs, such as seedlings and

leaves, and those dealing with resting or senescent organs such as tubers and fruits. Seedlings, owing to the fact that for many reasons they form admirable subjects for experiment, have received considerable attention from time to time. A good deal of the earlier work was carried out with a view to ascertaining the optimum temperature for respiration, but so far, no satisfactory conclusions have been reached with regard to this point. Although increases in temperature up to values in the neighbourhood of 45° C. are accompanied by corresponding initial increases in respiration intensity, these high initial rates are not always maintained. This point is well illustrated in Fig. 4, which is taken from the work of Fernandes and shows the effect on the respiration intensity of four-day-old pea seedlings, of changing the temperature from an initial value of 25° C. to various other experimental values. The operation of the time factor is well shown in this figure; it will be seen that raising the temperature of the seedlings to values higher than about 30° C. to 35° C. results in a falling off in respiration rate with time from the initial maximum rate for the temperature under consideration. For temperatures between 0° C. and 45° C., increase in temperature results in an increase in this initial respiration intensity, but temperatures above 45° C. result in a progressive lowering of the initial rate (cf. curves for 50° C. and 55° C. in Fig. 4). It would appear then that we must probably consider a temperature in the neighbourhood of 30° C. as the optimum for the experimental material under consideration, as at this temperature there occurs no falling off in the respiration intensity with time. A complication entering into work of this kind, when seedlings in the early stages of their development are used, results from the fact already described on p. 31, that the respiration intensity of seedlings germinating under certain conditions does not follow a level course, but shows

an initial rise and subsequent fall. Owing to the changes in germination rate produced by different temperatures, the time and duration of this so-called 'grand period of respiration' will be affected by temperature, so that it may influence the form of

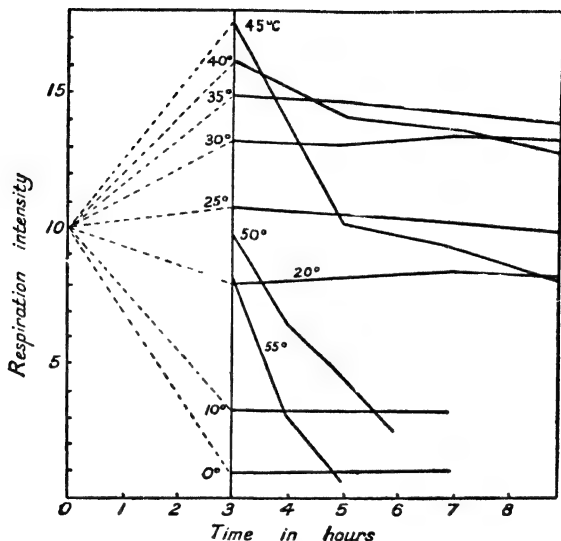



Fig. 4.—Curves showing the comparative rates of respiration of four-day-old seedlings of *Pisum sativum* as affected by different temperatures and related to time

(*After* Fernandes)

temperature-effect graphs similar to those shown in Fig. 4, causing up-grades or down-grades which are apt to be erroneously interpreted. This has probably happened in the case of some published work on the lines under discussion.

Another aspect of the question is that involving determinations of the temperature coefficient of the respiratory process. Such determinations are made

with a view to exploring the possibilities of the connexion between respiration and known chemical reactions which obey the Van't Hoff rule, that is, reactions, the velocity of which is approximately doubled or trebled by a rise in temperature of 10° C. The temperature coefficient is denoted by the symbol Q_{10} and is the ratio of the rate of the reaction or process at one particular temperature to its rate at a temperature 10° C. lower.

The operation of the time factor as outlined above often renders the determination of temperature coefficients for respiration, with any degree of certainty, very difficult ; in fact, at temperatures above about 30° C. attempts to make such determinations are probably of doubtful value. At temperatures between 0° C. and 30° C., in cases where the respiration intensity remains reasonably constant, estimations of the temperature coefficient may be of considerable value. In this connexion we have a Q_{10} value of 2·5 obtained by Clausen for wheat, lupin seedlings and syringa flowers between 0° C. and 20° C., and 2·1 by Blackman and Matthaei for cherry-laurel leaves over a range of 16° C. to 45° C. Gerhart working on the respiration of strawberry fruits obtained a Q_{10} value of 2·5 between 5° C. and 25° C., but with temperatures above 25° C. it was impossible to arrive at any consistent value for the coefficient. Values of the same order of magnitude for the temperature coefficient of the respiration of germinating peas between 0° C. and 20° C. are indicated by the data of Kuijper and Fernandes.

An interesting effect of temperature upon respiration intensity is that described by Müller-Thurgau and Schneider-Orelli for the potato. They investigated the changes in the course of carbon dioxide output by potato tubers brought about by maintaining them at various temperatures for eight-hour periods, and then recording their rates of carbon dioxide evolution over periods of fourteen days at a tem-

perature of 19° C. The effect of this treatment is indicated in Fig. 5. It will be seen that the previous heating produces an initial increase in respiration intensity which subsequently falls off. In the case of those tubers which were heated to temperatures higher than 38° C. the falling off ceases while respiration intensity is still higher than it was before the heating was carried out. In other words, heating potato tubers for eight hours at temperatures of

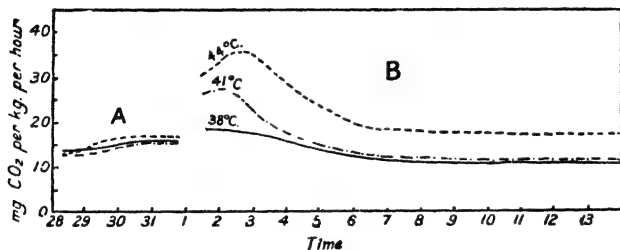



Fig. 5.—Curves showing the course of respiration intensity of potato tubers at a temperature of 19° C., at A, before, and B after they had been kept for eight hours at 38° C., 41° C. and 44° C. respectively. The experiment extended from 28 March to 14 April.

(*After* Müller-Thurgau and Schneider-Orelli)

41° C. and 44° C. produced a permanent increase in respiration intensity at normal temperatures.

The effect of temperature on the respiration of stored apples formed part of the already mentioned investigation of Kidd and West (cf. p. 34). The three curves given in Fig. 3 show the course of the respiration intensity during the period of senescence of apples when stored at temperatures of 2·5° C., 10° C., and 22·5° C. respectively. These investigators found that one effect of increased temperature was a shortening of the senescent period beginning with the removal of the fruit from the tree and ending with their death from fungal attack. Another effect was an increase in respiration intensity (see Fig. 3).

This influence of temperature on senescent drift and carbon dioxide output was found to be such that the total amount of carbon dioxide liberated from the time of gathering to the time of death was approximately the same, no matter at what temperature the fruit was stored. It was also found that the amount of dry matter lost by the fruit during the senescent period was approximately constant, regardless of temperature.

Light.—Various attempts have been made from time to time to determine the effect of light on respiration intensity, the results of which are inconclusive or contradictory. The present position, therefore, with regard to this question is that light appears to have either very little or no direct effect on respiration. Indirectly, however, in the case of plant organs containing chlorophyll, light may have a very considerable effect on respiration intensity through its influence upon the supply of respiratory substrate resulting from photosynthesis. Light may have an apparent effect on respiration rate owing to its action in causing a decomposition of organic acids with a resulting liberation of carbon dioxide, as mentioned in connexion with the metabolism of succulent plants on p. 22.

Concentration of Oxygen.—Although the normal respiration in plant organs is dependent upon an adequate supply of oxygen for its continuance, until recently the widely accepted view has been that considerable variations in the concentration of this gas in the surrounding atmosphere may occur without causing any change in respiration intensity. In the case of low concentrations, figures were put forward by Stich indicating that the percentage of oxygen in the atmosphere may be reduced to a low value before a change to anaerobic respiration is indicated by the already mentioned rise in the respiratory quotient (see p. 24). It seems likely, however, that this view may undergo considerable

revision in the near future as Blackman states that recent work carried out in his laboratory indicates that the carbon dioxide production of plant tissues 'varies with every alteration of the oxygen concentration of the environment', and further that 'carbon dioxide production in the complete absence of oxygen may be much higher, or much lower than in air'. In the case of the fruit of the apple, Blackman has found that in pure nitrogen the carbon dioxide production is high, and that as oxygen is admitted it falls off rapidly until the oxygen concentration is about 5 per cent. to 9 per cent. Further gradual increase in the oxygen concentration up to 100 per cent. results in a corresponding steady increase in carbon dioxide output.

Concentration of Carbon Dioxide.—Increased concentration of carbon dioxide in the atmosphere brings about very marked depression in the respiratory process. Although it has been known since the time of de Saussure that high carbon dioxide concentrations in the surrounding air are injurious to plants, the effect of this factor on plant respiration was not clearly brought out until the publication of the researches of Kidd. He examined the effect of various concentrations of carbon dioxide in air, on both the oxygen intake and carbon dioxide output of germinating seeds of white mustard, and also on the carbon dioxide output of cherry-laurel leaves. In the case of germinating mustard seeds, the data resulting from Kidd's experiments are set out in Table X.

It will be seen that the depressing effect of concentrations up to 50 per cent. carbon dioxide varies roughly with the square root of the concentrations.

With regard to the experiments upon leaves in this connexion, owing to technical difficulties the results obtained are less conclusive, but from the data obtained it appears that the inhibiting effect of high carbon dioxide concentrations is confined to

the 'floating' respiration, the 'protoplasmic' respiration being unaffected.

TABLE X

THE RETARDING INFLUENCE OF INCREASED CONCENTRATIONS OF CARBON DIOXIDE UPON THE RATE OF NORMAL RESPIRATION IN GERMINATING WHITE MUSTARD SEEDS, MEASURED BY CO_2 PRODUCTION AND OXYGEN CONSUMPTION

(*From* Kidd)

	Concentrations of carbon dioxide initially present					
	0%	10%	20%	30%	40%	80%
After 14 hours :						
c.c. CO_2 gain / c.c. O_2 loss	58/71	48/57	38/49	33/45	26/38	17/32
Respiratory quotient .	0·82	0·84	0·77	0·73	0·69	0·53
After 40 hours :						
c.c. CO_2 gain / c.c. O_2 loss	173/197	158/185	96/122	75/104	61/97	41/90
Respiratory quotient .	0·87	0·85	0·75	0·72	0·63	0·45

Conducted in dim diffuse light. 20 per cent. oxygen present initially in each experiment. 15 gm. of seed set dry on 50 c.c. damp sand and 10 c.c. tap water in each experiment. Results obtained from analyses. Temperature of experiments, 25·5° C. by thermostat.

Kidd also experimented with green peas, and suggested that the dormancy of seeds is brought about by the presence of high carbon dioxide concentration in the tissues resulting from restriction by the testa of the free passage of gases. This theory is probably nearer the truth than others previously advanced, which give lack of oxygen as the cause of dormancy.

Ionized Air.—Before leaving the question of the changes in respiration intensity produced by variations in the composition of the atmosphere, one other point seems worthy of mention, namely, the

effect of ionized air. It has been found that plants, or parts of plants, respire more actively in air that has been ionized by means of radio-active substances than they do in air that has not been so treated. As the gases of the atmosphere are to some extent ionized during daytime by the action of sunlight, this factor probably has some small effect on the respiration of plants growing under natural conditions.

Sugar.—The concentration of sugar in culture solutions in which fungi are growing has a marked effect on the respiration intensity of these plants. This fact has been demonstrated by Maige and Nicolas and by Kosinski. Various sugars were used in the experiments of Maige and Nicolas, who found that, generally, respiration intensity increased with increased sugar concentration up to a point when plasmolysis set in, when respiratory rate decreased. It has also been shown that the respiration of etiolated leaves which are poor in sugar, is considerably increased by immersing the petioles of the cut leaves in sugar solution.

Various Substances.—A considerable amount of attention has been paid by plant physiologists to the changes in the intensity of plant respiration produced by various chemical substances. Weak solutions of mineral salts and inorganic acids appear to cause an increase in respiration intensity. As an example of this, Kosinski found that amounts of zinc sulphate, ferric chloride and manganese chloride varying from 0·0005 per cent. to 0·06 per cent. when added to cultures of *Aspergillus niger* caused increases of 15 to 38 per cent. in the output of carbon dioxide by the fungus. Nitric acid and potassium nitrate have been found to increase the respiratory quotient of leaves, while Wehner, measuring intake of oxygen, observed that the aquatic moss *Fontinalis* respires more actively in a mixture of 0·0001 N nitric acid and 0·1 N sodium nitrate than in corresponding solutions of either of these individual compounds.

The effects of various organic poisons on the respiratory processes of plants are not without interest. The effect of chloroform upon the respiration of cherry-laurel leaves has been investigated by Miss Irving, who found that small doses cause an increase in respiration intensity which may persist so long as the application of the substance is continued. Medium doses cause an initial increase in intensity which is followed by a decrease to much below normal, the larger the dose the more rapid the decrease. Strong doses of chloroform result in a rapid fall in respiration rate to zero without any initial increase occurring. Other workers have carried out similar investigations with various plant organs and using a variety of poisons including ether, cocaine, morphine, quinine, chloral hydrate, caffeine, ethyl bromide, formaldehyde, acetone and ethyl alcohol. Broadly speaking, the results described are very similar to those outlined above. Although vague theories have been advanced in attempts to account for these effects of poisons on plant cells, no satisfactory conclusions have been so far arrived at; the matter will, therefore, not be discussed further.

The Effect of Wounding on Respiration.—It has long been known that when a plant organ is wounded an increase in respiration intensity results. Böhm in 1887 called attention to the fact that when potatoes are cut they exhibit an increased output of carbon dioxide. In 1891, Stich published a more comprehensive account of the phenomenon, having investigated it in other plants in addition to the potato. He also showed that when a potato was cut into two parts, and the cut surfaces joined together again by means of neutral gelatin, the resulting increase in respiration intensity was less than when the cut surfaces were left exposed to the air. Five years later Richards described wounding experiments upon potatoes, carrots, beet, and the hypocotyls and

roots of *Vicia* and *Cucurbita*, and various leaves. In all cases he obtained, after injury, respiration rates which varied in intensity and duration with the character of the tissue involved and with the extent of the wounding. This increased respiratory activity usually reached a maximum within two days, after which it fell gradually until an approximately normal rate was resumed. In the case of bulky tissues, for example potato and carrot, there occurred during the first two or three hours a sudden increase followed by a rapid decrease in the amount of carbon dioxide evolved. This was due to the escape from the cut surfaces of gas previously enclosed in the tissue.

In order to investigate the cause of this increase in respiration intensity which follows wounding, Hopkins measured the respiration of cut potatoes and also determined the variations in sugar content which occurred in the cut tuber. He found that this increased by from 53 to 68 per cent. of the original sugar content, and that the maximum occurred several days after wounding, after which it slowly fell. He also found that the maximum sugar content was reached after the time of maximum respiration intensity had been passed; this appeared to be due to the fact that suberisation of the wound occurred, causing accumulation of carbon dioxide in the tissue, thereby bringing about a lowering of the respiration rate. In passing, it may be suggested that accumulated carbon dioxide in tissues may frequently be a limiting factor in respiration intensity.

Before concluding this chapter it may be mentioned that other forms of stimulation have been found to cause an increase in respiration intensity. As examples of this we have the increase in respiration rate exhibited by the carpels of flowers after pollination has taken place, and similar increases in the case of roots undergoing curvature as a result of geotropic response.

CHAPTER III

ANAEROBIC RESPIRATION

IT is well known that most animals, when deprived of oxygen, cease to respire and die in consequence. The behaviour of plants is in marked contrast to this. When removed from a supply of oxygen a plant which normally respires aerobically continues to give out carbon dioxide, and the production of this gas may continue for a longer or shorter time according to the plant material. If anaerobic conditions are maintained for too long a time the plant suffers injury and may be killed in consequence, but if the absence of oxygen is not too prolonged, the plant, on return to a normal atmosphere, behaves quite normally and is found to be perfectly healthy. The actual time for which an aerobic plant can withstand anaerobic conditions without injury depends upon various conditions such as temperature and food supply. It was found by Chudiakow in 1894 that maize seedlings in absence of oxygen die in 24 hours at 18° C. and in 12 hours at 40° C., while it has been stated that apples and pears remain uninjured for months in an atmosphere of pure nitrogen or pure hydrogen.

The first definite observation on record of the evolution of carbon dioxide by plants in absence of oxygen was made in 1797 by William Cruickshank, Chemist to the Ordnance and Surgeon of Artillery. We have thought it of interest to quote his own description of one of his experiments, for it was none

other than the ordinary laboratory method of demonstrating anaerobic respiration which has been performed and observed by many thousands of students in succeeding decades, although pea seeds are usually substituted for barley grains.

'*January* 20*th*. A quantity of barley, soaked as in former experiments, was introduced into a jar filled with and inverted over mercury. At the expiration of 12 days a very considerable quantity of gas was produced, at least five or six times the bulk of the barley; but nothing like vegetation was perceivable. The gas on examination was found to consist of carbonic acid, being entirely absorbed by lime-water. The barley had not the least sweet taste, nor did it appear to have undergone any sensible change.'

This experiment, and other early ones, are perhaps not to be regarded as highly critical, especially in regard to the complete exclusion of oxygen and micro-organisms, but later observations showed, without a doubt, that the conclusion derivable from these early experiments was correct, and that an evolution of carbon dioxide by aerobic plants in absence of oxygen is a general phenomenon. This evolution of carbon dioxide in absence of oxygen was described as 'intramolecular' by Pflüger, who in 1875 observed the phenomenon in the frog, the idea involved in this term being that the carbon and oxygen of the exhaled carbon dioxide must come together within the molecules of the substance of the animal. This term was carried over into plant physiology by Pfeffer, but it is not a very happy one, and the expression 'anaerobic respiration' introduced by Kostychev in 1902 is now almost universally employed by writers in English, although 'intramolekulare Atmung' is still used by some German writers.

In establishing the general existence of anaerobic respiration, and in confirming the earlier observation

that along with the evolution of carbon dioxide, alcohol is produced, Pasteur and his pupils Lechartier and Bellamy played a prominent part sixty years ago. This discovery of alcohol as a product of anaerobic respiration at once suggested a comparison of the latter process with yeast fermentation in which the products are also carbon dioxide and alcohol. Although Sachs did not accept the view that there is a connexion between these two processes, and indeed considered 'that the formation of alcohol in the absence of oxygen is an abnormal process throughout, and has nothing to do with ordinary respiration', the evidence since Pasteur's time for the similarity of anaerobic respiration and alcoholic fermentation has grown rather than diminished, as will appear from a consideration of the evidence presented in the next chapter.

However, anaerobic respiration does not always result in the accumulation of ethyl alcohol in the tissues, and only exceptionally is the amount found that which we should expect if the sugar is completely respired to carbon dioxide and alcohol. The equation representing this reaction, namely,

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

indicates that equal molecular quantities of alcohol and carbon dioxide are produced. Since the molecular weights of these substances are respectively 46 and 44 the quantities should also be almost equal in weight ($CO_2/C_2H_5OH = 0{\cdot}96$). An approximate equality of output of the two products was actually observed by Godlewski and Polzeniusz in 1901, who obtained a ratio of CO_2/C_2H_5OH varying between 0·975 and 1·096, a relation confirmed by Nabokich in 1903, who obtained a value of 0·984. Subsequent observations, especially by Kostychev and Boysen Jensen, have, however, shown that this agreement with theory is by no means general, as an inspection of the following table shows.

TABLE XI

Ratio of Alcohol to Carbon Dioxide formed in Anaerobic Respiration

Species	Organ	C_2H_5OH/CO_2	Observer
Mucor racemosus .		0·99	Kostychev
Aspergillus niger .		0·92	,,
Psaliota campestris	fruit body	0·00	,,
Pisum sativum .	cotyledons	0·65	Boysen Jensen
,, ,, .	,,	0·81	,, ,,
Acer platanoides .	leaves	0·58	Kostychev
Prunus padus .	,,	0·51	,,
Syringa vulgaris .	,,	0·56	,,
Tropaeolum majus	,,	0·45	Boysen Jensen
,, ,,	,,	0·24	,, ,,
,, ,,	,,	0·17	,, ,,
Daucus carota .	root	1·02	Kostychev
,, ,, .	,,	0·91	Boysen Jensen
,, ,, .	,,	0·86	,, ,,
,, ,, .	,,	0·72	,, ,,
Brassica rapa . .	,,	0·49	Kostychev
Lepidium sativum	seedlings	0·57	,, ,,
Sinapis sp. . .	,,	0·60	Boysen Jensen
,, ,, . .	,,	0·32	,, ,,
Pyrus malus, var. *sinap* (sweet apples)	fruit	0·82	Kostychev
Pyrus malus, var. *Anton* (sour apples)	,,	0·42	,,
Citrus aurantum (orange)	,,	0·70	,,
Vitis vinifera (green grapes)	,,	0·86 0·74	Boysen Jensen
Vitis vinifera (blue grapes)	,,	0·95	,, ,,
,, ,,	,,	0·88	,, ,,
,, ,,	,,	0·81	,, ,,
,, ,,	,,	0·74	,, ,,
Solanum tuberosum, var. *Magnum bonum*	dormant tuber	0·07	Kostychev
Solanum tuberosum, var. *Magnum bonum*	sprouting tuber	0·00	,,
Solanum tuberosum, var.	tuber	0·07	Boysen Jensen
,, ,,		0·02	,, ,,
,, ,,		0·00	,, ,,
,, ,,		0·00	,, ,,

From this table it will be observed that among higher plants, only exceptionally is the amount of alcohol found in anaerobically respiring tissues equal to that of the carbon dioxide evolved. In potato tubers ethyl alcohol may be completely absent. Two explanations of this state of affairs are possible. Either the alcohol is produced in the amount indicated by the equation given above and is immediately utilized in some secondary reaction, or the respiration process does not always take the same course, and other products are formed besides, or instead of, ethyl alcohol. There is not sufficient evidence to decide between these two possibilities.

According to Palladin, the formation of alcohol in anaerobic respiration only takes place when the supply of carbohydrate is sufficient. If this is not the case, carbon dioxide is produced by the breaking down of some other cell constituent, and some other product may result.

It is significant in this connexion that Kostychev and Afanassjewa found that *Aspergillus niger*, when grown on carbohydrate media under anaerobic conditions, gave a yield of alcohol which was within a few per cent. of the theoretical amount. On the other hand, when this mould was grown on a peptone medium, no alcohol was produced. This is apparently correlated with the fact that on the latter medium the fungus forms no zymase, the enzyme complex which, as is well known, brings about the splitting of sugar into alcohol and carbon dioxide, whereas on a medium containing sugar these enzymes are produced. It therefore seems likely that the process of anaerobic respiration of *Aspergillus niger* may vary according to whether the mould is grown on sugar or peptone media. There is thus a possibility of different types of anaerobic respiration. On the other hand, when, in anaerobically respiring tissue, the amount of ethyl alcohol produced is not equivalent to the quantity of carbon dioxide formed, the fact

that *some* ethyl alcohol usually accumulates rather suggests that ethyl alcohol is first formed and then used in a secondary reaction. There is, however, the possibility that some intermediate product, instead of producing ethyl alcohol, may give rise to some other substance or substances. Such an intermediate product might be acetaldehyde, the presence of which has been demonstrated in anaerobically respiring tissues and which is now commonly regarded as a precursor to the production of alcohol in anaerobic respiration (see Chapter IV).

Various investigators concluded that among other products of anaerobic respiration are to be included various organic acids such as formic, acetic, propionic and oxalic. Little, however, is known about the origin of such substances in anaerobic respiration.

It is generally supposed that the capacity of aerobic plants to remain alive for a time while deprived of an oxygen supply is directly related to their power of anaerobic respiration which supplies a certain amount of energy. The energy released in this case is, however, small in comparison with that produced during normal respiration. Thus in aerobic respiration, for every molecule of sugar completely oxidised to carbon dioxide and water, 674 calories are released, whereas the energy released in the splitting of one molecule of hexose to carbon dioxide and alcohol is usually stated to be 25 to 28 calories. Or put in another way, since six molecules of carbon dioxide result from the complete oxidation of one molecule of hexose, and only two molecules of carbon dioxide from the fermentation of one molecule of hexose, for every molecule of carbon dioxide formed in aerobic respiration about 112 calories are released as compared with only about 13 calories per molecule of carbon dioxide released in anaerobic respiration. An inspection of Table XII indicates that the rate of anaerobic respiration of a plant or organ is rarely as high as its rate of aerobic respiration, so that on

exclusion of oxygen the energy produced in respiration falls usually to one-ninth or less of its previous value. Only in ripening fruits, as so far observed, is the ratio of the energy released in anaerobic respiration to that released in aerobic respiration likely to be a little higher than this.

Varying external conditions appear to affect anaerobic respiration, as far as can be judged from published results, in very much the same way as they affect aerobic respiration. As regards temperature, the most reliable data concerning the influence of this factor on anaerobic respiration are probably those of Fernandes published in 1923 and dealing with germinating peas respiring in an atmosphere of hydrogen. Fernandes' results with seedlings 21 hours old are shown graphically in Fig. 6. A comparison with the results for seedlings respiring aerobically, shown in Fig. 4, at once reveals how similar are the temperature effects in aerobic and anaerobic respiration. It will be observed that up to 30° C. the rate of respiration remains practically constant with time, but that above this temperature a time factor very obviously operates. The rates of respiration in air and hydrogen are compared in the following table.

TABLE XII

EFFECT OF TEMPERATURE ON GERMINATING PEAS 21 HOURS OLD IN AIR AND IN HYDROGEN

(*From* Fernandes)

Temperature	Respiration in hydrogen	Respiration in air
0	0·87	0·93
10	2·35	2·5
20	6·08	6·32
25	12·9	10·47
30	15·4–16·8	13·2–16·8

These values give average temperature coefficients (Q_{10}) over the range of temperatures 0° to 30° of about 2·67 in the case of respiration in hydrogen and about 2·52 for respiration in air. The values

are of the same order of magnitude and the differences are almost certainly within the limits of experimental error. Probably no conclusion is justified from

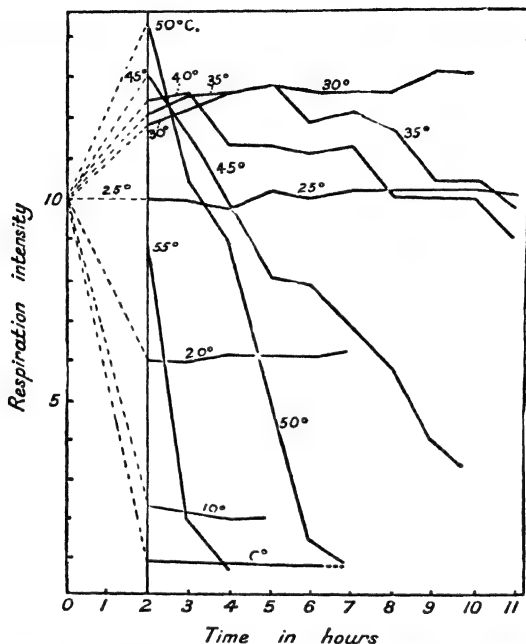



FIG. 6.—Curves showing the comparative rate of respiration of 21-hour-old seedlings of *Pisum sativum* as affected by different temperatures and related to time, when respiring in an atmosphere of hydrogen

(*After* Fernandes)

this similarity apart from a supposition that a purely chemical process determines the rate of respiration whether under aerobic or anaerobic conditions.

Poisons have been found to affect aerobic and anaerobic respiration to about the same extent.

Karlsen compared the aerobic and anaerobic respiration of wheat seedlings subjected to the action of various volatile poisons, namely, ether, benzene and ethyl alcohol. The relative effects of each poison upon the course of respiration in air and in nitrogen respectively were found to be similar.

Quite a number of attempts have been made to discover a quantitative relation between the intensities of aerobic and anaerobic respiration of the same organ. The first of these was made by Wortmann in 1880 on germinating seeds of *Vicia faba;* he found the rates of carbon dioxide evolution in air and in a Torricellian vacuum to be the same. Although subsequent measurements confirmed this result for this material, they show that there is in general no such agreement between the intensities of anaerobic and aerobic respiration. In Table XIII are shown the values published by Pfeffer in 1885 for the ratio of respiration intensity in hydrogen to respiration intensity in air $\left(\frac{R_h}{R_a}\right)$ for a number of plant organs.

From these values it will be observed that the intensity of anaerobic respiration is less than that of aerobic respiration, as measured by rate of carbon dioxide output, in every case except that of germinating seeds of *Vicia faba,* while the actual ratio of the two intensities varies within wide limits. Subsequent determinations of the ratio by other observers have given similarly divergent results, the values varying between 0·177 and 0·181 recorded for seedlings of *Sinapis alba* by Pfeffer in 1885, and 1·3 and 1·1 found for green grapes by Boysen Jensen in 1923.

Boysen Jensen's work on this subject contains observations which suggest that the numbers found for the ratio of anaerobic to aerobic respiration may not always have any definite value; for he shows that in two cases examined, that of leaves of

TABLE XIII

RATIO OF ANAEROBIC TO AEROBIC RESPIRATION
(*From data published by* Pfeffer)

Species	Organ	$\frac{R_h}{R_a}$
Vicia faba	germinating seed	1·03 (mean of four determinations)
Triticum vulgare	seedling	0·49
Cucurbita pepo	,,	0·35
Sinapis alba	,,	0·18
Brassica napus	,,	0·25
Cannabis sativa	,,	0·34
Helianthus annuus	,,	0·33
Lupinus luteus	,,	0·24
Heracleum giganteum	unripe fruit	0·42
Abies excelsa	young leafy shoot	0·077
Orobanche ramosa	flowering stem	0·32
Arum maculatum	,, ,,	0·615
Ligustrum vulgare	leafy shoot	0·816
Lactarius piperatus	fruit body	0·32
Hydnum repandum	,, ,,	0·256
Cantharellus cibatius	,, ,,	0·666
Beer Yeast (on lactose)		0·310

Tropaeoleum majus at 13° C. and seedlings of *Sinapis alba* at 16° C., the rate of respiration in hydrogen did not remain constant but fell off very definitely and considerably with time. This behaviour of the *Sinapis* seedlings is indicated graphically in Fig. 7, which shows that the average rate of respiration during the fourth hour in hydrogen was only about a quarter of that during the first hour. The behaviour of the *Tropaeoleum* leaves was very similar. It will be observed from Fig. 7 that in the case of *Sinapis* seedlings, on replacing hydrogen by air the rate of respiration rises to above its original value. In the absence of information regarding the course of aerobic respiration during development, no conclusion can be drawn concerning this increased rate.

Considerable attention has been directed by Parija to the change in rate of carbon dioxide evolution by apples on transference from aerobic to anaerobic conditions. Parija's investigation formed the second of a series of *Analytic Studies of Respiration* carried out in F. F. Blackman's laboratory and published in 1928. The investigation of Blackman and Parija on the respiration of apples in air indicated that the fruit they used, belonging to the variety Bramley's Seedling, did not exhibit uniform behaviour

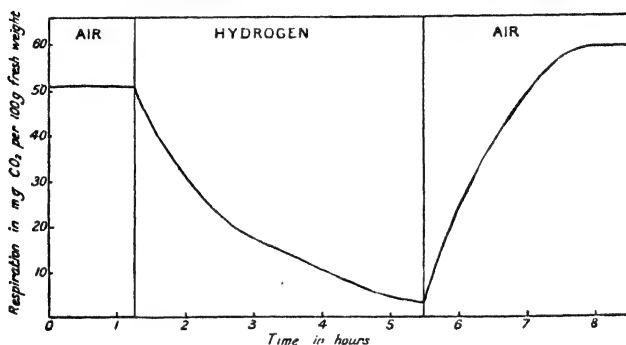



FIG. 7.—Graph illustrating the effect on carbon dioxide output of seedlings of *Sinapis alba* when changed from an atmosphere of air to one of hydrogen and *vice versa*
(*From data published by* Boysen Jensen)

in regard to the course of their respiration, but it was concluded that the differences could be explained on the view that the apples belonged to three physiological classes which ripened at different rates. The respiration of these apples on transference to anaerobic conditions, in this case an atmosphere of pure nitrogen, is also dependent on the physiological class. In the case of slow-ripening apples, transference to nitrogen always brings about an immediate *increase* in the carbon dioxide output which rises to a maximum in a few hours and then falls

rapidly to the level of respiration in air, at which level it continues for a shorter or longer time, finally declining rapidly and regularly. A typical example is shown in Fig. 8. With apples of the more rapidly maturing class (or classes) the rate of respiration rises immediately on substitution of nitrogen for

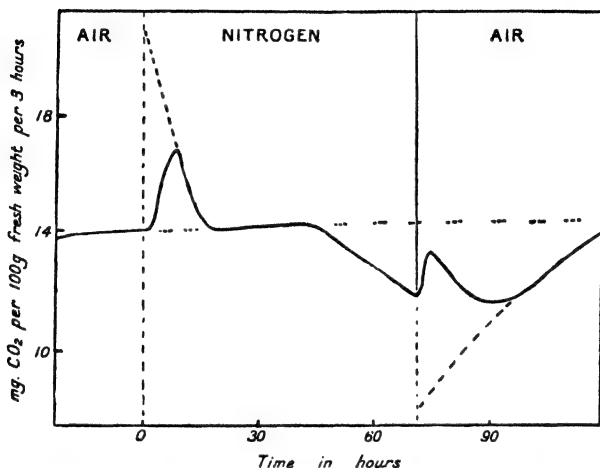



Fig. 8.—Graph illustrating the effect on carbon dioxide output of Bramley's Seedling apples belonging to the slow-ripening class, produced by change from an atmosphere of air to one of nitrogen, and *vice versa*

(*After* Blackman)

air, and then declines slowly with fluctuations to a level well above that which would obtain in air. A typical case of this type is exhibited graphically in Fig. 9.

On re-transference to air the carbon dioxide output follows a fluctuating course, first rising, then falling and rising again, but ultimately reaching the normal rate of output for air, although sometimes not until the lapse of two days. Actually, on trans-

ference of the apple from air to nitrogen, the observed change in carbon dioxide output lags behind the change in respiration intensity, owing to the time required for the respiratory carbon dioxide to diffuse from the tissues of the apple to the surrounding gas in the plant chamber. It is therefore reasonable to conclude that the actual rate of respiration in

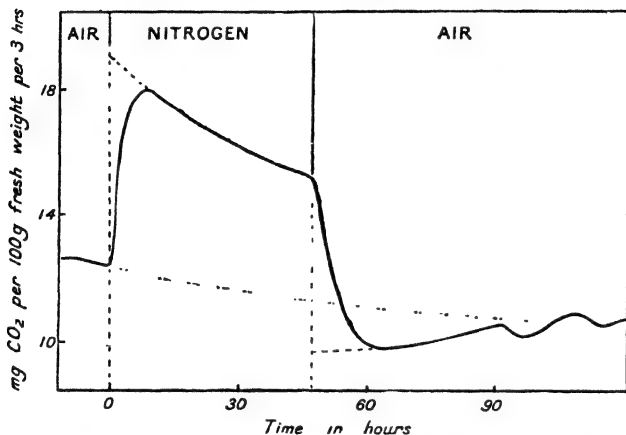



Fig. 9.—Graph showing effect of an atmosphere of nitrogen on the carbon dioxide output of Bramley's Seedling apples of the rapidly ripening class

(*After* Blackman)

nitrogen is highest at the moment of transfer to that gas and falls continually as shown by the broken lines in Figs. 8 and 9. This initial value of respiration rate in nitrogen is found to be either about 1·5 or 1·33 times the rate in air immediately before the replacement of air by nitrogen. As we shall see later, the value of this ratio is thought by Blackman to be of considerable importance in shedding light on the series of reactions involved in respiration.

This higher rate of carbon dioxide output in

nitrogen as compared with the rate in air appears to be exceptional as far as observations go at present. It is, however, possible that high rates of anaerobic respiration are characteristic of senescent fruits. Thus G. R. Hill in 1913 observed that rates of anaerobic respiration as measured by carbon dioxide output were as high, or higher, than those of aerobic respiration, in the case of senescent grapes, cherries, and blackberries. It has already been mentioned that Boysen Jensen in 1923 obtained similar results in the case of the first of these fruits. Immature fruit does not, as far as observations go, behave in this way. An important point connected with the above fact is that since three times as much sugar is required to produce a definite quantity of carbon dioxide anaerobically as is required to produce it aerobically, it follows that the destruction of sugar or other respiratory substrate must proceed three or more times as rapidly in absence of oxygen as in its presence.

Recently some researches have been carried out by one of the authors [1] on the changes produced in the respiration of germinating seeds when they are successively subjected to atmospheres of air, nitrogen and again air. The results obtained show that germinating seeds when they are placed in nitrogen exhibit a respiratory behaviour which differs markedly from that observed by Blackman in the case of apples. The species used were *Ricinus*, *Helianthus* and *Cucurbita* as representatives of fat-storing seeds and *Lathyrus*, *Zea* and *Fagopyrum* as carbohydrate-storing seeds.

In the case of the fat-storing seeds the change from air to nitrogen produces an immediate and rapid fall in the rate of carbon dioxide output. This initial fall is followed by a more gradual fall in carbon dioxide production and this last fall continues throughout the anaerobic period.

[1] See Leach and Dent, 1934 and 1936.

The change from nitrogen to air produces an opposite effect, the carbon dioxide output increases first of all rapidly and then more slowly as the normal aerobic respiration of the seedlings is resumed.

The carbon dioxide output of carbohydrate-storing seeds shows a similar rapid initial fall when the change from air to nitrogen is made. It also shows a similar rapid rise and subsequent resumption of a normal course when the seedlings are transferred from nitrogen to air. The respiratory behaviour however of carbohydrate seeds in nitrogen during the period immediately following the initial fall in carbon dioxide production is peculiar. After this initial fall, the output of carbon dioxide first rises for a time and then again falls, this last fall being continued throughout the rest of the anaerobic period. It would appear from this that, with carbohydrate-storing seeds, the change from aerobic to anaerobic conditions brings into existence a fresh source of carbon dioxide, but as the substrate which produces this new carbon dioxide output is limited in quantity and is only produced in presence of oxygen, the rise in respiratory activity produced by it can be maintained only for a short time.

The final gradual fall in anaerobic carbon dioxide production shown by all the seeds used would further appear to indicate that in all cases the substrate for anaerobic respiration is dependent in the first place upon the presence of oxygen for its production.

Measurements of the respiratory quotients of the experimental seedlings were made and these have thrown some additional light on the effect of anaerobic conditions. With all the seedlings used, the respiratory quotient falls rapidly for a time when the change from nitrogen to air is made. This fall indicates a high rate of oxygen absorption by the seedlings, a fact which indicates that during the period in nitrogen some readily oxidisable substance accumulates in

their cells. The fall in the quotient is followed by a rise which continues until the normal value for the particular seedling is reached.

The numerical relationships between the final respiration rate in air and the initial respiration rate in nitrogen when these various seedlings are transferred from air to nitrogen, are shown in Table XIV and may be compared with the values obtained by Blackman for apples. (Cf. p. 62.)

TABLE XIV

THE RELATIONSHIP BETWEEN RESPIRATION IN AIR (OR) AND RESPIRATION IN NITROGEN (NR) OF YOUNG SEEDLINGS

Seedling	$\frac{NR}{OR}$
Lathyrus odoratus	0·22
Fagopyrum esculentum	0·35
Zea mais	0·25
Helianthus annuus	0·52
Cucurbita pepo	0·40
Ricinus communis	0·51

An examination of the figures given on the above table show that with carbohydrate seeds (*Lathyrus*, *Fagopyrum* and *Zea*) a change from respiration in air to respiration in nitrogen results in a reduction in the carbon dioxide output to one-third or less than one-third. In the case of the three fat-storing seeds the reduction is however not so great. In these, the relatively high values of $\frac{NR}{OR}$ may in part be due to an insufficient supply of oxygen, when the seedlings are respiring in air, to allow OR to attain its full value. A suggested reason for this is that oxygen, besides being used for respiration, is in fat-storing seeds used for the conversion of fat into carbohydrates.

Reference has been made in the preceding chapter

to the effect of oxygen concentration on respiration. It seems likely that in low concentrations of this gas, carbon dioxide may arise partly by aerobic and partly by anaerobic processes. Indeed, Blackman has attempted an analysis of the carbon dioxide output of apples in low oxygen concentrations into its aerobic and anaerobic components.

It has been suggested that anaerobic respiration may normally take place where diffusion of gases between the tissues and outer air is slow. Such a state of affairs may prevail in germinating seeds provided with testas of a low degree of permeability, in middle parts of bulky tissues such as large fruits, and various organs of succulent plants, in woody stems surrounded by a cork layer, and in organs submerged in water. In all such organs it is at any rate a possibility that the actual oxygen concentration in the respiring cells may be low, while in addition there may be a partial accumulation of carbon dioxide so that the concentration of this is maintained at an unusually high level and so may retard respiratory activity. (Cf. p. 45.)

As regards bulky tissues such as large fruits, Gerber in 1896 held that anaerobic respiration always takes place in these and that through this process arise the alcohols, aldehydes, and esters which are normally present in such fruits. Devaux had, however, shown in 1891 by direct analysis, that the internal atmosphere of large cucurbitaceous fruits, as, for example, those of *Cucurbita maxima* and *C. melanosperma*, contains nearly as high a concentration of oxygen as is present in atmospheric air, percentages of this gas of 18·29 and 17·89 being found in these two species, respectively. Also Meirion Thomas has recently found only very small quantities of ethyl alcohol and acetaldehyde in freshly gathered apples, namely 0·006 per cent. by weight of the former and 0·0005 per cent. by weight of the latter. These observed facts suggest that, as far as

they go, very little anaerobic respiration is likely to occur in fleshy fruits, the aerating system being adequate to allow of rapid enough diffusion of gases for oxygen respiration to proceed normally. It is significant that Thomas found a considerable accumulation of ethyl alcohol in apples stored in an atmosphere of pure nitrogen, the percentage of alcohol rising to 0·17, 0·25 and 0·39 after 15, 23 and 38 days, respectively, at 1° C.

It should be noted, however, that Thomas and Fidler found there was a production of ethyl alcohol and acetaldehyde in old apples kept, not only in air, but in 100 per cent. oxygen. Denoting by the term zymasis the breakdown of carbohydrate with formation of these substances, they distinguish between anaerobic zymasis which takes place in absence of oxygen, and carbon dioxide zymasis which takes place under aerobic conditions if carbon dioxide is present in comparatively high concentration. The two types of zymasis differ in the amount of acetaldehyde produced, there being more of this formed under anaerobic conditions than under aerobic. The general conclusion drawn from these observations is that one of the features of senescence in the apple is a change in the metabolism of the fruit leading to the accumulation of ethyl alcohol, and, to a less extent, of acetaldehyde. Zymasis is retarded by oxygen, but as the fruit grows older a higher concentration of that gas is necessary to suppress alcohol production until ultimately it is not affected even in 100 per cent. oxygen.

The aerobic production of ethyl alcohol has also been observed by Gustafson in tomatoes, but in this fruit he did not find the difference in the acetaldehyde production under aerobic and anaerobic conditions observed by Thomas and Fidler in apples.

CHAPTER IV

THE MECHANISM OF RESPIRATION

The Connexion between Fermentation, Anaerobic Respiration and Aerobic Respiration

WE have seen that normal aerobic respiration consists broadly of the oxidation of carbohydrates or other organic material into carbon dioxide and water. Since this means the breaking down of a complex molecule containing at least six carbon atoms into carbon dioxide with but one, it is extremely unlikely that the respiratory process takes place in a single step. One of the aspects of an inquiry into the mechanism of the respiratory process is, therefore, the determination of the probable stages in the breaking down of the carbohydrate or other complex material utilized in respiration.

Further, we are well aware that under temperature conditions similar to those prevailing in a living organism no breaking down of carbohydrate into carbon dioxide and water takes place if we merely supply carbohydrate with oxygen. A second aspect of the inquiry into the mechanism of respiration is therefore concerned with an examination of the special conditions of the living cell which enable this katabolism to take place. Here obviously we have to consider the various oxidation systems, enzymatic and otherwise, which are known to be present in the cell, as well as enzymes which split off carbon dioxide from more complex substances, and to decide, as far as we are able, what con-

nexion, if any, they may have with the respiratory process.

These two aspects of our problem are, of course, intimately connected, for, from what we know of the characteristics of enzyme actions, it is at least a possibility that every stage in the process is catalysed by its own enzyme. It will therefore not always be possible to separate them in discussion.

While there is little direct evidence regarding the mechanism of the respiration process, a considerable amount of indirect information is available from fairly recent investigations on yeast fermentation. The value of this information in regard to the problem of normal aerobic respiration depends largely on (*a*) to what extent fermentation by yeast is identical with anaerobic respiration, and (*b*) the connexion between anaerobic and aerobic respiration. With regard to the former of these questions it has often been assumed that yeast fermentation and anaerobic respiration are the same process. There are several facts in favour of this view. In fermentation, hexose sugar is utilized and the same is frequently the case in anaerobic respiration ; where some other substance is utilized it is possible, and even probable, that the substance is first transformed into hexose sugar before it can be utilized for respiration. In both processes carbon dioxide is produced, while in many cases of anaerobic respiration the formation of ethyl alcohol has been demonstrated. Where this has not been done, and where the quantity of alcohol formed, relative to the carbon dioxide produced, is less than that formed in fermentation, the absence or shortage of this substance can be attributed to its utilization in some secondary reaction. Thus, as far as is known, both the substrate and products in anaerobic respiration are the same as those in alcoholic fermentation.

Of course, it does not follow that because the substrate and end products are the same, that these

latter have been produced by the same mechanism. There are, however, additional pieces of evidence suggesting that this is indeed so. The enzyme, or rather group of enzymes, known as zymase, which was already known to be the catalyst bringing about the breaking down of hexose to alcohol and carbon dioxide in yeast fermentation, was shown to be present in the cells of a number of higher plants by Stoklasa and Czerny in 1903. More recently there have been isolated from yeast a number of enzymes which in all probability form part of the zymase complex. Such are glycolase, ketonaldehydemutase and carboxylase. Further reference will be made to these later. It will suffice here to mention that all these enzymes which play their respective parts in the various stages of yeast fermentation have also been isolated from various higher plants.

Again, Neuberg has shown that acetaldehyde is probably formed as an intermediate product in alcoholic fermentation, and, by the addition of a sulphite to the fermenting liquor, can be fixed as aldehyde sulphite and so caused to accumulate. Now Neuberg and Cohen have similarly succeeded in fixing aldehyde in the anaerobic respiration of various fungi, while Neuberg and Gottschalk have similarly fixed aldehyde formed in the anaerobic respiration of pea seeds. Previous to this, in 1913, Kostychev, Hübbenet, and Scheloumov had demonstrated the formation of acetaldehyde in anaerobically respiring poplar flowers.

One other point of resemblance between yeast fermentation and anaerobic respiration that remains to be mentioned is perhaps not so significant. Fermentation of sugar by means of expressed yeast juice or dried yeast is accelerated by the addition of soluble phosphate, while addition of phosphate to the tissues of higher plants killed in various ways also leads to an increased rate of carbon dioxide production. The reason for the latter has, however,

been the subject of much controversy. One school, represented in particular by Zaleski, L. Ivanov, and N. Ivanov, holds that the phosphate does actually accelerate respiration, the other, of which the chief exponent was Kostychev, holds that where phosphate brings about an increased rate of carbon dioxide output from dead tissues, it is merely due to the effect of the phosphate in increasing the alkalinity of the medium. However, Lyon found that phosphate accelerates both aerobic and anaerobic respiration.

But, even if we leave the question of the effect of phosphate on respiration as doubtful, the evidence already cited in favour of the view that alcoholic fermentation is the same process as anaerobic respiration is very strong. We are justified, in the absence of any definite evidence to the contrary, in assuming, at least provisionally, that fermentation of sugar by yeast and anaerobic respiration follow the same course.

The relationship between aerobic and anaerobic respiration is not so clear. That a close connexion exists between these processes was first suggested by Pflüger for the case of certain animals which could carry on respiration anaerobically for a time. His idea was that the respirable material was first broken up anaerobically into carbon dioxide and easily oxidizable products, the latter then being oxidized by atmospheric oxygen to carbon dioxide and water.

Although such authorities as Nägeli and Sachs held the evolution of carbon dioxide in absence of oxygen to be a pathological phenomenon due to injury resulting from absence of oxygen, and therefore to have no connexion with normal respiration, the view of Pflüger was taken over by Pfeffer into plant physiology. He suggested that ordinary aerobic respiration takes place in two stages, the first, a splitting of sugar by a number of steps into alcohol and carbon dioxide, the second, the oxidation by

atmospheric oxygen of the alcohol, or some other product formed in one of the steps of anaerobic respiration, into carbon dioxide and water. The first of these stages is independent of oxygen and is in fact the process called anaerobic respiration.

This theory found little support at the time, and Pfeffer himself later appeared to give it up. Its lack of support was largely due to two causes. In the first place no constancy was found in the ratio between the intensities of anaerobic and aerobic respiration for different plant organs. In the second place a series of experiments by Diakanov demonstrated that anaerobic respiration of certain fungi could only take place on saccharine media, although aerobic respiration took place in a wide range of other media.

With regard to the first of these difficulties in the way of accepting Pfeffer's hypothesis it was argued that if anaerobic respiration is the first stage of normal respiration, the intensities of the two processes should bear a constant relation to one another. The numbers given in Table XIII, Chapter III, indicate that this is not so, and that the ratio of the respiration rate in hydrogen to that in air may vary widely from plant to plant and organ to organ. This difficulty in the way of accepting the Pfeffer theory is, however, only apparent. If the theory were correct there is no reason why the ratio of the intensities of anaerobic respiration and aerobic respiration should be constant. In the first place, under anaerobic conditions the rate of respiration will depend, at the beginning, on the concentration of the sugar; later, if alcohol accumulates, the latter will tend to retard the process more and more, not merely in accordance with the law of mass action, but because of its toxic effect on the respiring cells. We have already noted that it is highly probable that alcohol accumulates to different degrees in different plant organs. We may therefore add that

it seems likely that the rate of anaerobic respiration in different plants and different plant organs is a function of the amount of alcohol accumulated at any time, and that this varies very considerably from plant to plant. Under aerobic conditions, provided the resulting alcohol is oxidized as soon as it is formed (and the absence of alcohol in the tissues would support this view), the rate of aerobic respiration will suffer no retardation on account of accumulation of products.

The second difficulty, which was the outcome of Diakanov's experiments, was proved by Kostychev to be also without foundation. Kostychev repeated Diakanov's work and showed that his experimental results were largely vitiated by the toxic action of the products of metabolism under anaerobic conditions. When this toxic action was eliminated no distinction could be found between saccharine and non-saccharine nutrients such as glycerol, mannitol, and lactic acid when used as substrates for anaerobic respiration.

Thus these particular objections to the theory of a close connexion between anaerobic and aerobic respiration disappear. Further, although there is no direct evidence in favour of such a connexion, there are several facts which indirectly suggest that it exists. These are as follows:

1. Anaerobic respiration of normally aerobic plants when deprived of oxygen, appears to be a universal phenomenon. It is true that Lyon failed to observe the evolution of carbon dioxide from *Elodea* in absence of oxygen, but this appears to be an isolated and exceptional case.

2. The enzyme complex zymase, which, as we know, is concerned in the anaerobic splitting off of carbon dioxide from sugar, appears to be universally present in plant cells. It would thus appear that the process in which zymase is concerned is part of the normal respiratory mechanism of the cell. Since the action

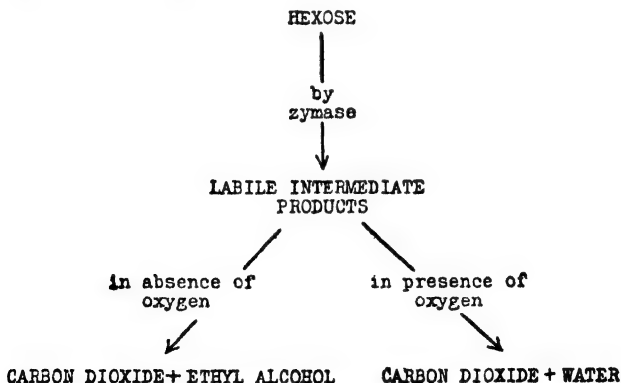
of zymase is not affected by oxygen, the absence of alcohol production in presence of oxygen cannot be explained as due to the inhibition of zymase.

3. If the first stage (or stages) of normal respiration consists of the anaerobic production of easily oxidizable substances, a period of anaerobic respiration should lead to the accumulation of such substances. Subsequent transference to aerobic conditions should then, owing to the increase in concentration of the substrate for the oxidation process, result in a rate of respiration above the normal. Such an increased rate of respiration after a period of anaerobiosis was observed by Maquenne in 1894 and has subsequently been recorded by Palladin and other observers. Where such an increase is not recognizable it may sometimes be accounted for by the toxic effect of the products of anaerobiosis.

4. It has been put forward by Kostychev that the essential plant-oxidizing systems, to which reference will be made shortly, are incapable of oxidizing sugars, but are able to oxidize substances present in fermented sugar solutions. It seems possible, however, that the oxidation observed might be explained as due to presence of sugars in a labile form which could be more readily oxidized than sugars in the ordinary stable form. The significance of this will become clearer when the question of the transformation of stable into labile sugar as a preliminary stage in respiration is discussed.

5. It has been mentioned that acetaldehyde is in all probability an intermediate product in anaerobic respiration. It is therefore significant that Klein and Pirschle have demonstrated the formation of acetaldehyde during normal aerobic respiration.

There is thus a considerable weight of evidence in favour of the theory of a close connexion between anaerobic and aerobic respiration. However, the simple view which considers anaerobic respiration to be the first stage of aerobic respiration is unlikely to

be correct, for ethyl alcohol is even less easily oxidized than hexose sugars. Further, there is no evidence of a mechanism in plant cells in general, capable of bringing about the oxidation of ethyl alcohol. Pfeffer's alternative theory has been urged by Kostychev and meets this difficulty. According to this theory, anaerobic respiration or alcohol fermentation itself takes place in several stages, a formation of labile intermediate substances produced by the action of zymase preceding the production of alcohol. Under anaerobic conditions, these intermediate substances pass over into alcohol, but when oxygen is present the labile substances are oxidized to carbon dioxide and water. From what has been stated previously it would appear that acetaldehyde might be such an intermediate substance yielding alcohol or becoming completely oxidized according to conditions. This theory may be represented schematically thus:

Current views of the mechanism of respiration may be regarded as an elaboration of this theory, although they have actually developed more or less independently of it.

During recent years the validity of the hypothesis that aerobic and anaerobic respiration are connected in all plants has been called in question by Boysen Jensen. In 1923 this investigator pointed out that in certain plant material, including *Tropaeolum* leaves, *Sinapis* seedlings, *Aspergillus niger* and *Penicillium glaucum*, the ratio of the rate of anaerobic to the rate of aerobic respiration sinks below 1/3 without the material suffering injury. Now since one molecule of hexose sugar yields six molecules of carbon dioxide by complete oxidation, and two molecules of carbon dioxide by fermentation or anaerobic respiration, it follows that in such cases anaerobic processes do not split up enough sugar to account for the whole of the aerobic respiration. More recently the same author has pointed out that D. Müller has prepared an enzyme, glucose oxidase, from *Aspergillus niger*, which can oxidize glucose to gluconic acid directly without the intervention of zymase, so that breaking down of hexose by the latter is not a necessary preliminary for oxidation. Further, E. Lundsgaard has found that zymase is easily paralysed by monoiodoacetic acid, so that baker's yeast treated with this substance in suitable concentration has very little fermenting power; it can, however, oxidize sugar to carbon dioxide and water, so that again zymase is not necessary in the chain of reactions involved in the complete breaking down of sugar. It is to be noted that glucose oxidase is rather resistant to the action of monoiodoacetic acid.

From a consideration of these facts, Boysen Jensen concludes that some organisms are able to oxidize sugar directly without its being first subjected to splitting by zymase.

Enzymes Concerned in Respiration

From what has been written above it will appear that the complete respiration of hexose under aerobic

conditions may involve breaking up of the sugar molecule as in yeast fermentation followed by an oxidation process. We may therefore expect two sets of enzymes to be concerned in respiration: those involved in yeast fermentation and those bringing about oxidations in the plant. It will therefore be in place briefly to consider these two sets of enzymes.

(*a*) *Fermentation Enzymes*

In 1897 E. Buchner obtained from yeast a product which brought about fermentation of sugar into alcohol and carbon dioxide without the intervention of the living cell. To this product, which possessed the general properties of an enzyme, the name zymase was given.

Subsequent work leaves no doubt that zymase is a mixture of enzymes, though at the moment it cannot be said how many enzymes are present in it. There is a certain amount of evidence that some or all of the following enzymes, which may be concerned in the fermentation process, are either present in the zymase complex or accompany it.

1. Hexosephosphatase, which is concerned in the formation of fructose diphosphate from hexose and phosphate.

2. Phosphatase, which hydrolyses fructose diphosphate into fructose and phosphate, and which is generally regarded as distinct from hexosephosphatase which brings about the synthesis of fructose diphosphate.

3. Glycolase, which acts on fructose diphosphate, or more probably the fructose produced by the action of phosphatase, to give methyl glyoxal (pyruvic aldehyde) $CH_3.CO.CHO$.

4. Ketonaldehydemutase, which acts on methyl glyoxal to give lactic acid.

5. Aldehydemutase, which acts on aldehydes to give esters or their constituent acids and alcohols.

6. Carboxylase, which acts on α-ketonic acids, such as pyruvic acid, to give aldehydes by the

splitting off of carbon dioxide. Thus pyruvic acid yields acetaldehyde and carbon dioxide.

As mentioned earlier, the presence of some of these enzymes has also been demonstrated in plants other than yeast. Thus Bodnár has obtained indications of hexosephosphatase in pea meal, Neuberg and his co-workers have demonstrated the presence of ketonaldehydemutase in several plants, while carboxylase has been demonstrated in seeds of *Pisum sativum*, *Vicia faba* and *Lupinus luteus* by Zaleski and Marx, and in potatoes and beetroot by Bodnár.

In addition to enzymes, other substances are necessary for fermentation. Definitions of enzymes vary somewhat, but if we accept that one which describes an enzyme as a soluble thermo-labile colloidal organic catalyst formed in a living organism, then in addition to the zymase complex a crystalloidal thermo-stable organic substance is necessary, for such a substance can be separated from zymase by dialysis, thus rendering it inactive. This is the substance called co-zymase, and on adding it to the colloidal zymase residue the latter is again rendered active. Without going further into the details of co-zymase, it should be mentioned that the existence of such a co-enzyme has also been demonstrated in higher plants. It is, however, not clear how far the various components of zymase require the presence of a co-enzyme for their action. The available evidence does, however, suggest that a co-enzyme is as necessary for anaerobic respiration as for yeast fermentation.

Phosphate is also generally held to be essential for yeast fermentation, as the first stage in that process appears to be the formation of fructose diphosphate. Whether this is also the case in anaerobic respiration generally does not appear to have been settled, although, as we have already observed, it has been stated that the presence of

phosphate accelerates the rate of both aerobic and anaerobic respiration.

(b) Oxidation Enzymes and Other Systems

Although a very great deal of research has been performed on oxidation systems in living organisms, knowledge of these systems and ideas of their mechanism both appear to be in a somewhat confused state and no two authorities on oxidizing systems in living cells classify them in the same manner. For our purpose, however, we may consider four groups of enzymes concerned in oxidation, although this does not necessarily exhaust the oxidizing systems involved. These four groups of enzymes are catalase, peroxidase, oxidase (including oxygenase), and dehydrase (also called dehydrogenase and oxidoreductase). A part in the respiration process has been ascribed to each of the four enzymatic systems mentioned above, as well as to other substances or systems which cannot be regarded as enzymes in the strict sense. A brief statement of the characteristics of these various systems will, therefore, not be out of place here.

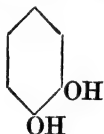
1. *Catalase.*—This enzyme is widely distributed throughout the plant kingdom and may possibly be present in all plants. It brings about the decomposition of hydrogen peroxide into water and oxygen. As, however, the oxygen is given off in the molecular state, it has been stated that catalase is not an oxidizing enzyme, and its general function appears to be merely the destruction of hydrogen peroxide, which may be produced as a by-product in some stage of respiration.

2. *Peroxidase.*—This enzyme brings about oxidation in presence of hydrogen peroxide and certain organic peroxides, but the mechanism of its action is quite different from that of catalase. Like catalase, it may perhaps be universally present throughout the plant kingdom, for it has been found in nearly all higher plants where it has been sought. At

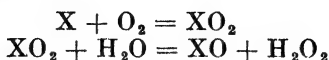
one time the presence of peroxidase was recognized by its power to turn a tincture of guaiacum gum blue in presence of hydrogen peroxide, the blue colour being due to the formation of an oxidation product of guaiaconic acid present in the gum. Besides guaiaconic acid a number of other phenolic compounds such as pyrogallol, catechol, *p*-cresol and benzidine are also oxidized in presence of peroxidase and hydrogen peroxide.

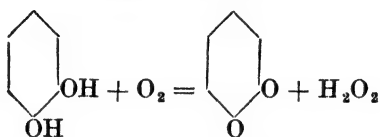
It has been usual to think of peroxidase as splitting hydrogen peroxide into water and " active " oxygen, the latter then combining with the substance oxidized.

It is also possible that some preparations described as peroxidase are not enzymatic according to the definition of this enzyme cited on p. 78. Thus Keilin describes a substance cytochrome, isolated from muscle and yeast, as a peroxidase, and this substance, a derivative of haematin, appears to be thermo-stable and possibly not colloidal. Reference is made to cytochrome and some other non-enzymatic oxidizing systems later.

3. *Oxidase.*—The oxidases have been defined as enzymes which oxidize by means of free oxygen. The best known plant enzyme of this type is that known as oxygenase or catechol oxidase. This enzyme catalyses by means of molecular oxygen the oxidation of compounds possessing the ortho-dihydroxy grouping

characteristic of catechol. It does not appear to be certain at the moment what substance is actually formed by the oxidation of the catechol or related compound. According to one view, by addition of a molecule of oxygen, a catechol peroxide, for example, is

produced which in presence of water is decomposed into hydrogen peroxide and some other oxidation product of catechol. This reaction might be summarized thus:

$$X + O_2 = XO_2$$
$$XO_2 + H_2O = XO + H_2O_2$$

On the other view hydrogen is transferred from the catechol to molecular oxygen to form orthoquinone and hydrogen peroxide thus:

$$\text{OH} + O_2 = \quad \text{O} + H_2O_2$$
$$\text{OH} \qquad\qquad\qquad \text{O}$$

Oxygenase does not appear to be present in all species. Of 300 species of flowering plants examined by Mrs. Onslow, 63 per cent. were found to contain oxygenase. In these oxygenase-containing plants an aromatic compound with the catechol grouping is also present, and no species has been discovered which contains oxygenase without the catechol compound, while there appear to be few, if any, species containing a catechol compound without oxygenase.

The tissues of oxygenase-containing plants are also characterized by changing to a reddish-brown, brown or black colour on injury or exposure to chloroform vapour. This colour change is attributed to the oxidation of the catechol compound by the action of the oxygenase and probably also to subsequent secondary oxidations.

Since all plants presumably contain peroxidase, the hydrogen peroxide formed by the oxygenase action, can, with the former enzyme, bring about such secondary oxidations.

Mention should here be made of an enzyme which is present in yeast and which has been regarded by

some workers as an oxygenase, although its reactions are not the same as those of the oxygenase of the higher plants. To this oxidizing enzyme of yeast the name 'indophenol oxidase' has been given on account of its action on the so-called indophenol reagent (an alkaline mixture of *p*-phenylenediamine and *a*-naphthol), which on treatment with the enzyme yields an oxidation product indophenol blue.

Warburg supposes that the absorption of oxygen by cells is due to a reaction between molecular oxygen and an iron compound, probably a haematin derivative, which he calls the respiratory enzyme; and he supposes that oxidation processes in cells are only brought about through the action of this iron compound. Warburg's theory is based principally on the effect of carbon monoxide, cyanides and other substances in inhibiting reactions catalysed by iron compounds. Warburg's respiratory enzyme, according to Keilin, is simply the indophenol oxidase.

4. *Oxido-reductase.*—Much prominence has been given in recent years to the theory of oxidation put forward by Wieland. According to this theory oxidation is brought about by the 'activation' of the hydrogen in the substance oxidized (the 'hydrogen donator'), by the action of a catalyst, dehydrase, the activated hydrogen being transferred to another substance (the 'hydrogen acceptor'), which is thereby simultaneously reduced. Enzymes bringing about this activation of hydrogen have also been termed dehydrogenase, and, since both an oxidation and reduction are involved, oxido-reductase. From what has been said above, peroxidase might be regarded as an oxido-reductase activating the hydrogen in the compound to be oxidized and transferring it to hydrogen peroxide which is thereby reduced to water. Oxygenase, also, on the second view of its action mentioned on p. 81, might be regarded as activating the hydrogen of catechol and transferring it to molecular oxygen, which is thereby

reduced to hydrogen peroxide. Onslow and Robinson tested this view, but could obtain no support for it.

However, apart from these particular cases, a number of enzymes have been found which catalyse the oxidation of one compound in the presence of another which is reduced. The best known enzyme of this type is the nitrate-reducing enzyme found in potato tuber and a number of other plants. This enzyme in the presence of aldehyde brings about the reduction of nitrates to nitrites, the aldehyde being oxidized to acetic acid.

Of particular interest in this connexion are oxido-reductases which catalyse the so-called 'Cannizzaro reactions'. In a Cannizzaro reaction two molecules of an aldehyde in the presence of water are transformed into one molecule each of the corresponding acid and alcohol, one aldehyde molecule being thus oxidized to the acid while the other is reduced to the alcohol.

5. *Non-enzymatic Oxidizing Systems.*—A number of substances which can act as catalysts in oxidation processes and which do not fulfil the characteristics of enzymes have been isolated from plant and animal cells. Among the most noteworthy of these are glutathione and cytochrome.

Glutathione.—This was isolated by Hopkins in 1921 from animal cells, and subsequent tests suggest that it is widely distributed throughout the plant kingdom. According to the most recent investigation of its chemical nature it appears to be a tripeptide of cysteine, glutamic acid and glycine. In the presence of a hydrogen acceptor the glutathione is oxidized and the acceptor reduced; in the presence of a hydrogen donator the oxidized glutathione is reduced and the donator oxidized. Glutathione could thus bring about the oxidation or reduction of various substances in the cell.

Cytochrome.—Keilin has isolated a number of

haematin compounds from yeast and animal cells which have definite oxidative qualities. These substances belong to the iron-porphyrin group, and are therefore chemically related to haemoglobin and chlorophyll, although their actual composition has not been worked out. In yeast Keilin distinguished four such compounds, an unbound protohaematin and three haematin compounds forming the *a′*, *b′*, and *c′* components of cytochrome. To these substances Keilin attributes the thermostable peroxidase reaction of yeast and aerobic bacteria; indeed, he states that in all organisms examined, cytochrome and other haematin compounds are responsible for the thermostable peroxidase reactions.

Like glutathione, cytochrome can undergo reduction, and the reduced cytochrome, oxidation. The oxidation and reduction can be easily observed in living cells spectroscopically, for both the oxidized and reduced substance show characteristic and different absorption bands. Keilin regards cytochrome, or at any rate its *a′* and *c′* components, as definitely concerned in the respiratory mechanism. Further reference to his views will be made later.

Respiration Chromogens.—This term was applied by Palladin to denote certain substances widely distributed throughout the plant kingdom and which can be extracted from plant tissue by boiling water. When to such an extract peroxidase and hydrogen peroxide are added, a red, or more rarely violet, colour is produced owing to the oxidation of the so-called respiratory chromogen, the pigment formed being called a respiration pigment. According to Palladin, these substances play a part in the respiration process. By the action of an oxidase the chromogen is oxidized by atmospheric oxygen to the respiration pigment. This then acts as a hydrogen acceptor and is reduced to the chromogen, the hydrogen donator, which is presumably an intermediate product in the respiration process, being

thus oxidized. The respiration chromogens and pigments are thus similar in their behaviour to reduced and oxidized glutathione and to reduced and oxidized cytochrome.

According to Mrs. Onslow, Palladin's respiration pigments are the orthoquinones produced by the action of oxygenase on catechol.

The Stages in the Respiration Process

We are now in a position to examine more easily the question of the actual stages in the respiration process. From what has already been stated it will appear that in all probability the course of both anaerobic and aerobic respiration is the same up to a certain stage, after which the course of the process depends upon the presence or absence of oxygen and on other conditions. At the same time it should be emphasized that these suggested stages in the respiration process are no more than probabilities. In other words they amount to a theory of the course of respiration, which, with further work, may have to be modified to a less or greater degree.

The course of aerobic respiration may conveniently be considered in three main parts, though each part may contain several stages. These main parts are:

(1) the production of active hexose from the normal sugar or other material used in respiration,
(2) the breaking down of the active hexose by means of enzymes of the zymase complex,
(3) the oxidation of the intermediate products formed in (2).

Some workers would consider the respiration process to begin with (2), but whether (1) is included or not is merely a matter of definition.

1. *The Production of Active Hexose*

The numbers given in Table III on p. 20 serve as an example of the very general phenomenon that

seeds on germination lose part of their dry matter, a loss which must be ascribed to respiration. Such a loss of substance is naturally to be observed in all respiring plant material kept under such conditions that assimilation is prevented. Thus parts of autotropic plants such as isolated roots, tubers of potato or Jerusalem artichoke, and rhizomes of *Iris*, can easily be shown to lose dry weight as a result of respiration. But since many seeds germinate normally in the dark, since, moreover, they exhibit a high respiratory activity, and since they also frequently contain a considerable quantity of material which might be expected to furnish a substrate for respiration, they form particularly suitable objects for researches on the utilization of substances in respiration. The same is to some extent also the case in ripening fruits, in which assimilation is generally non-existent or negligible, and in which, therefore, the course of respiration can be followed, both as regards gaseous exchange and utilization of material, without the complication of concomitant assimilatory processes.

In Chapter II it has been mentioned that in different seeds different material may be utilized during germination according to the nature of the food reserve. Thus in wheat and maize there is a loss of carbohydrate, whereas in seeds that store large quantities of fat it is this that chiefly disappears. In others, again, protein may be utilized. It must therefore be supposed that any of these, and probably other substances as well in other plants, may provide a substrate for respiration.

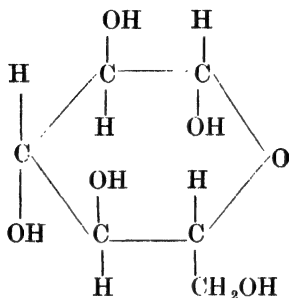
For several reasons it is convenient to consider the case of those plants in which the material utilized in respiration is a carbohydrate. In the first place, apart from seeds, plants which store carbohydrate appear to be in a great majority; in the second place it would seem most probable that in practically all assimilating organs where sugar is formed this is

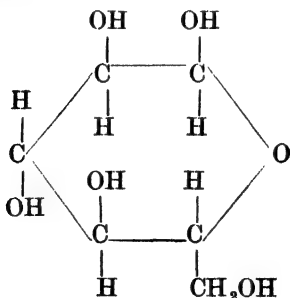
likely to be utilized for respiration; while thirdly, in yeast fermentation which, as we have seen, is extremely similar to, if not identical with, anaerobic respiration in normally aerobic plants, sugar forms the substrate for respiration. If the supposition usually made with regard to the close connexion of aerobic and anaerobic respiration is correct, it is obviously a possibility that when fat, protein or some other organic substance is used for respiration, such substance has first to take part in some reaction giving rise to sugar. We must, however, leave this point open and confine our attention to the usual case where the substrate for respiration is undoubtedly carbohydrate.

The carbohydrate reserves in plants are various, but are *chiefly* cellulose, starch, sucrose and the two hexose sugars glucose and fructose. Cellulose and starch are present as insoluble parts of the cell, and the presence of enzymes which hydrolyse them to glucose renders it extremely probable that this hydrolysis takes place before these reserves can be utilized. The presence of invertase, which converts sucrose to glucose and fructose, also suggests that sucrose is not utilized as such. Such considerations, along with the apparently identical character of the respiratory process in plants independent of the carbohydrates they contain, lead us to the conclusion that probably in all plants in which carbohydrate disappears in respiration, the actual substrate is a hexose sugar or sugars; glucose, fructose, or both.

It will be necessary at this point to consider the constitution of the sugars. While a number of different structural formulae have been ascribed to the various sugars during the last forty years, the modern views of Haworth are now practically universally accepted. According to him, the molecule of ordinary glucose and of other sugars of the aldose series is best represented as a six-atom ring com-

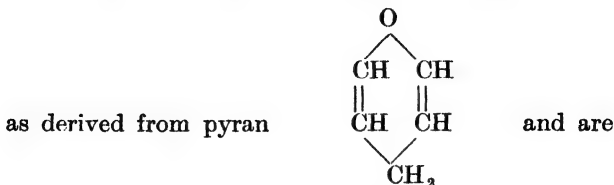
prising five carbon atoms and one oxygen atom, the sixth carbon atom forming part of a $-CH_2OH$ group which constitutes a side chain to the ring. Ordinary glucose exists in two stereoisomeric forms, α and β, of which the latter may be represented by the formula

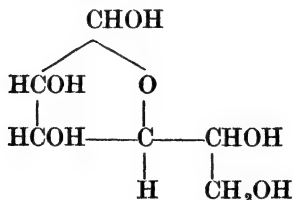
```
              OH     H
              |      |
   H         C ---- C
   |        /  |     |  \
   |       /   H     OH  \
   C                      O
   |  \     OH     H     /
   |   \    |      |    /
   OH    C ------- C
         |         |
         H         CH2OH
```

In this sugar the hydrogen atoms are regarded as lying alternately above and below the plane of the ring. In α-glucose the position of the hydrogen atom and the hydroxyl group attached to one of the carbon atoms is reversed so that this stereoisomer is represented by the formula

```
            OH     OH
            |      |
            C ---- C
   H       /|      | \
   |      / H      H  \
   C                   O
   |  \    OH     H   /
   OH  \   |      |  /
         C ------ C
         |        |
         H        CH2OH
```

These two isomers are both obtainable, and in solution can pass by mutarotation from one to the

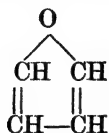
other, while both give stable derivatives such as the well known α- and β-methyl glucosides.

Sugars possessing such a formula can be regarded as derived from pyran

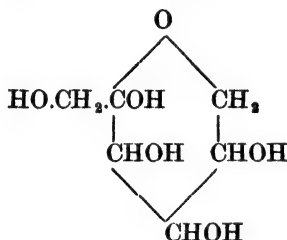
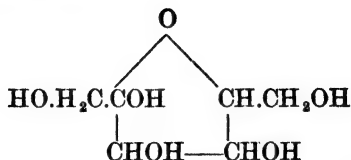
O
CH CH
|| ||
CH CH
CH_2

and are therefore termed pyranose sugars.

There are, however, a number of derivatives of glucose in which sugar appears to be present in a different form, that is, the atoms appear to be in some other arrangement. The first of these compounds to be recognized was γ-methyl glucoside and subsequently a number of other derivatives of glucose have been obtained in which the sugar part of the molecule has the same structure. Moreover, α and β forms of some, at least, of these derivatives of glucose have been isolated. Their chemical behaviour is such as to indicate that they are built up from a sugar having a five-atom ring and to which the name γ-glucose is given. The formula ascribed to this sugar is

This sugar does not appear to have an independent existence, but since it enters into combination it is clear that the stable forms of glucose must be convertible into it under certain conditions, and that it

can be regarded as a labile form of ordinary glucose. Such labile or γ-sugars are said to belong to the furanose series on account of their relation to furan

```
       O
      / \
    CH   CH
    ||   ||
    CH---CH
```

Not only do sugars of the aldose series exhibit γ-forms, but those of the ketose series do also. Of the ketoses, one in particular, fructose, is almost universally present in plants, free or in combination with glucose as cane sugar. The formula of ordinary free fructose can be written as

and of γ-fructose as

```
                  O
                /   \
HO.H₂C.COH       CH.CH₂OH
        |         |
       CHOH------CHOH
```

The γ-form of fructose, like that of glucose, has never been obtained free, but it is in this condition that fructose occurs in combination in plants, for in both inulin and sucrose the fructose part of the molecule is in the γ-form. The free sugar on the

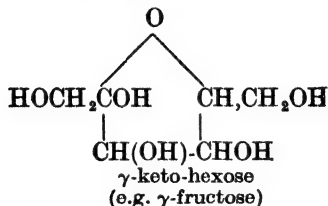
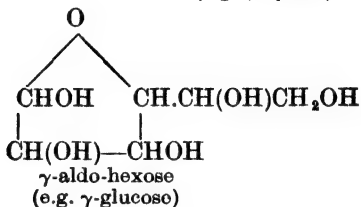
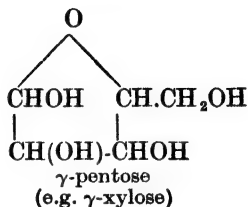
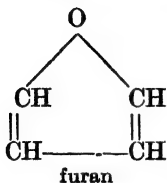
other hand is in the normal six-atom (pyranose) ring form.

Without going further into this question, it may be pointed out how closely related are the various γ-sugars, including not only those of the hexoses, but also of the pentoses. An inspection of the following formulae will make the relation clear.

furan

γ-pentose (e.g. γ-xylose)

γ-aldo-hexose (e.g. γ-glucose)

γ-keto-hexose (e.g. γ-fructose)

It seems, therefore, that a conversion of γ-fructose into γ-glucose or γ-pentose and vice versa in reactions in the plant involving hexose is not impossible or even improbable. It is well known that the pyranose forms of glucose and fructose readily pass from one to the other in alkaline solution. Further, it is clear that normal glucose is transformed to the

labile γ-form in the production of a number of glucose derivatives, as, for example, in the formation of γ-methyl glucoside from methyl alcohol and normal glucose, while not only can γ-fructose derivatives be produced from normal fructose, but the γ-fructose of sucrose is transformed to normal fructose (fructo-pyranose) on the inversion of the latter. Without definite evidence on the point, there is ground for supposing that a conversion in the plant of stable glucose and fructose into the labile and more highly reactive forms of these substances is likely.

Further support for this view is derived from a consideration of yeast fermentation, which, as we have seen, is either identical with, or closely related to, anaerobic respiration, which again has also in all probability a close connexion with aerobic respiration. Now fermentation by yeast juice or dried yeast is accelerated by the presence of phosphate, and it appears that this acceleration is due (1) to the formation of compounds of phosphoric acid and hexose, namely, hexose diphosphate and monophosphates, and (2) to the subsequent hydrolysis of the hexose-diphosphate into hexose and phosphoric acid. What part the monophosphates play is not clear. But whatever sugar is fermented, whether glucose, fructose or mannose, the diphosphate ester produced is always fructose diphosphate, and chemical evidence indicates that the fructose is present in the diphosphate in the γ-form.

No acceleration of fermentation by living yeast is observed on addition of phosphate. This may be due either to the presence in the cell of an optimal quantity of phosphate or to the cell membranes being impermeable to phosphate. It is, however, generally assumed that the first stage in yeast fermentation is the production of the hexose diphosphate ester and its subsequent hydrolysis. The obvious conclusion to be drawn as to the meaning

of this esterification and subsequent hydrolysis is that it effects the conversion of normal hexose into γ-fructose.

We may therefore regard the first stage in respiration as the formation of γ-fructose. In yeast this is brought about in two stages: (1) the formation of fructose diphosphate from a normal hexose sugar (glucose, fructose or mannose) and phosphate by the synthesizing action of hexosephosphatase, and (2) the hydrolysis of the fructose diphosphate by means of the enzyme phosphatase. In the higher plants the course may sometimes be the same, but where sucrose is present and utilized in respiration it seems likely that the hydrolysis of the sucrose will give γ-fructose directly.

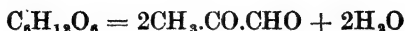
2. *Glycolysis: The Breaking Down of the Furanose Ring*

The splitting of hexose into alcohol and carbon dioxide in anaerobic respiration and fermentation has for long been considered to take place in a series of stages, for it involves the breaking down of a molecule containing six carbon atoms to molecules containing only two and one respectively. Various substances have been suggested as intermediate products, as, for example, lactic acid ($CH_3.CHOH.COOH$), methyl glyoxal or pyruvic aldehyde ($CH_3.CO.CHO$), glyceraldehyde ($CH_2OH.CHOH.CHO$), dioxyacetone ($CH_2OH.CO.CH_2OH$), acetaldehyde ($CH_3.CHO$) and formaldehyde ($H.CHO$). However, the work of Neuberg and his co-workers published during the last twenty years has rendered it almost certain that acetaldehyde is an intermediate product in fermentation, and so probably in respiration, as Kostychev supposed. As already mentioned, by addition of sulphite to the fermenting liquid, the aldehyde can be fixed and made to accumulate.

Neuberg has suggested a series of reactions by which the sugar may be broken down to acetaldehyde and while these must still be regarded as

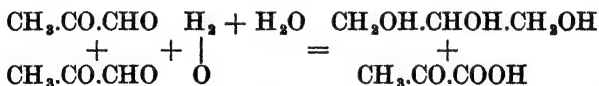
hypothetical they are at least credible and serve as an excellent working hypothesis. These stages are as follows:

i. The splitting of the hexose molecule into two molecules of methyl glyoxal with withdrawal of water:

$$C_6H_{12}O_6 = 2CH_3.CO.CHO + 2H_2O$$

Possibly glyceraldehyde may be first formed. It will be noticed that the production of two molecules of this substance from one molecule of γ-fructose (p. 90) involves relatively little change in the atomic arrangement obtaining in the latter beyond the breaking of the ring. There is, however, no direct evidence of the formation of glyceraldehyde.

It has already been pointed out that there is evidence for the presence of an enzyme glycolase [1] in yeast, which breaks down hexose into methyl glyoxal. So much was shown by Neuberg while Kobel and Scheuer have obtained evidence for the presence of such an enzyme in tobacco leaves.

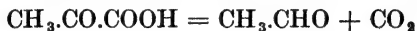
ii. This first stage in the breaking down of sugars, glycolysis in the restricted sense, is followed, on Neuberg's scheme, by a Cannizzaro reaction in which two molecules of methyl glyoxal are concerned, one being reduced to glycerol, the other being oxidized to pyruvic acid:

$$\begin{matrix} CH_3.CO.CHO & H_2 + H_2O & CH_2OH.CHOH.CH_2OH \\ + & + \; | & = \qquad + \\ CH_3.CO.CHO & O & CH_3.CO.COOH \end{matrix}$$

This action may be brought about by an oxido-reductase (cf. p. 82), possibly by the aldehydemutase mentioned on p. 77 or a similar enzyme. Neuberg

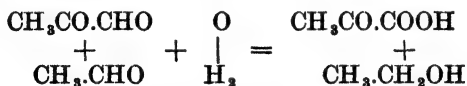
[1] The name glycolase was originally given to an enzyme which breaks down glucose to lactic acid in animal cells. This is probably a mixture of enzymes, including the yeast glycolase of Neuberg, and to it the name 'myozymase' has been given by J. B. S. Haldane.

has shown the presence, both in yeast and higher plants, of a ketonaldehydemutase which acts on methyl glyoxal, but the product is lactic acid, not glycerol and pyruvic acid. At present, therefore, there is no clear evidence for the presence of an enzyme catalysing this reaction. The fact, however, remains that glycerol can be detected as an intermediate product in fermentation, although the pyruvic acid cannot be, as it is presumably acted upon instantaneously.

iii. The pyruvic acid is now split into acetaldehyde and carbon dioxide :

$$CH_3.CO.COOH = CH_3.CHO + CO_2$$

This is the reaction catalysed by the enzyme carboxylase, now recognized as of wide and perhaps universal distribution in the plant kingdom.

4. Up to this point the course of respiration in aerobic and anaerobic conditions is probably the same. Under anaerobic conditions the next step is probably a second Cannizzaro reaction in which two aldehydes react together, a molecule of pyruvic aldehyde (methyl glyoxal) produced in stage 1 reacting with a molecule of acetaldehyde produced in stage 3 to give one molecule of pyruvic acid and one of alcohol :

$$\begin{array}{c} CH_3CO.CHO \\ + \\ CH_3.CHO \end{array} + \begin{array}{c} O \\ | \\ H_2 \end{array} = \begin{array}{c} CH_3CO.COOH \\ + \\ CH_3.CH_2OH \end{array}$$

The pyruvic acid is then immediately acted upon by carboxylase as in stage 3, but the ethyl alcohol in most cases accumulates. It will be observed that stage 2 may now be missed out of the general scheme as the pyruvic acid forming the substrate in stage 3 is being constantly re-formed in stage 4.

A somewhat different view of the stages in glycolysis has more recently been adopted by Embden and Meyerhof. They suggest that a molecule

of hexose diphosphate first reacts with another molecule of glucose and two of phosphoric acid to produce four molecules of phosphoglyceraldehyde $CH_2(PO_4H_2).CHOH.CHO$, which then, in a Cannizzaro reaction, give two molecules of phosphoglyceric acid $CH_2(PO_4H_2).CHOH.COOH$ and two molecules of phosphoglycerol (glycerophosphoric acid) $CH_2(PO_4H_2)CHOH.CH_2OH$. The latter now breaks down to pyruvic acid and phosphoric acid, and the pyruvic acid, by the action of carboxylase, breaks down to acetaldehyde and carbon dioxide in the same way as hypothesized in Neuberg's scheme. The theory of Embden and Meyerhof finds support in the production of phosphoglyceric acid in alcoholic fermentation.

3. *The Oxidation Process in Aerobic Respiration*

From what has been said already it would appear that the last stage in aerobic respiration is the oxidation of acetaldehyde or some other intermediate product or products by means of one of the oxidation mechanisms present in the plant. Here we are on anything but firm ground, and it must be realized that in spite of what is known of oxidizing systems in the plant, the actual course of this aerobic stage in respiration is still very obscure.

Attempts have been made to define the part played in respiration by the various oxidizing enzymes and other systems present in the living cell. Little has, however, resulted from these efforts. Of the oxidizing enzymes the catechol oxygenase does not seem to be universally present in plant cells, and since respiration appears to be a very uniform process throughout the plant kingdom it seems unlikely that to this enzyme can be ascribed a constant part in respiratory oxidation. Peroxidase and catalase both appear to be almost universally present in plant tissues. Whether the same applies to oxido-reductases cannot be said, as these enzymes have been scarcely examined critically. Various in-

vestigators have endeavoured to examine the extent to which the intensity of respiration varies with the concentration of the different oxidizing systems. Such investigations are beset with difficulties, but as far as they go they suggest that, in some cases at any rate, the intensity of respiration varies in the same direction as the concentration of catalase, from which it has been suggested that catalase is concerned in respiration. If this is so, what the function of catalase may be is certainly not clear. An obvious suggestion is that in respiration there is a production of hydrogen peroxide which is removed by catalase. The part played by the catalase in respiration would then probably be the removal of a harmful by-product, although the linking up of the catalase reaction with other reactions is possible. There is, however, no definite evidence available in the matter.

Most investigators have failed to find any relation between respiratory activity and concentration of other oxidizing enzymes. However, Keilin found that all the factors which inhibit the activity of indophenol oxidase of yeast also inhibit to about the same extent the respiratory activity of these cells as determined by Warburg and Meyerhof. Whether, in Keilin's words, 'this clearly shows that the indophenol or paraphenylene-diamine oxidase takes an essential part in the normal respiratory activity of the cells' may or may not be so, but the relation is used by Keilin to build up a theory of the respiratory mechanism in general.

Keilin, as we have seen, attributes to cytochrome an important function in the respiratory mechanism of the cell. According to Keilin's scheme dehydrases (oxido-reductases) activate the hydrogen of the molecules of the substrate. What this may be is not specified. It is presumably not sugar, and for the purposes of this discussion may be regarded as one or more of the intermediate products of anaerobic

respiration already mentioned. These substances thus become hydrogen donators. The hydrogen is 'accepted' by oxidized cytochrome *a′* and *c′* whereby the substrate is oxidized and the cytochrome reduced. The latter is then re-oxidized by the indophenol oxidase and so can again act as a hydrogen acceptor.

This is regarded as the main respiratory mechanism, but respiration can also take place without the intervention of oxidase by means of haematin and cytochrome *b′*. These substances are autoxidizable and do not require the presence of oxidase to be reconverted into the hydrogen acceptor. Respiration by means of these and other autoxidizable carriers is called by Keilin 'residual respiration' and, according to the nature of the cell, may form a larger or smaller part of the whole respiration.

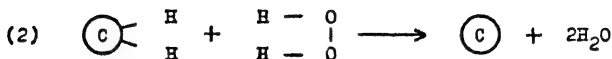
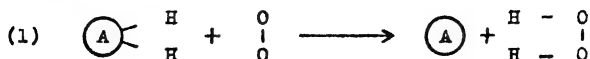
One of the weaknesses of Keilin's scheme is that, apart from observations on the wide distribution of the haematin pigments, it is largely based on observations on yeast, both as regards the oxidase and respiratory activity. Apparently Keilin would suppose that catechol oxygenases in the higher plants play the same part as the indophenol oxidase of yeasts, but there is no evidence that such is the case, while there is evidence that such oxygenases are wanting in quite a large number of plant species.

On the other hand, according to Harrison, who carried out a critical investigation on the indophenol reaction, there is no necessity for a specific indophenol oxidase to bring about the oxidation of either indophenol or cytochrome. Harrison himself proposes a scheme according to which reduced glutathione or some other hydrogen donator is oxidized in presence of molecular oxygen by removal of hydrogen with formation of hydrogen peroxide. The latter in presence of peroxidase is reduced to water and reduced cytochrome oxidized. Other reducing systems in the cell then bring about the

reduction of the cytochrome. The scheme may be represented thus:

$$(1)\quad (A){<}\begin{matrix}H\\H\end{matrix} + \begin{matrix}O\\|\\O\end{matrix} \longrightarrow (A) + \begin{matrix}H-O\\|\\H-O\end{matrix}$$

$$(2)\quad (C){<}\begin{matrix}H\\H\end{matrix} + \begin{matrix}H-O\\|\\H-O\end{matrix} \longrightarrow (C) + 2H_2O$$

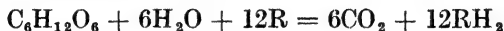
$$(3)\quad (B){<}\begin{matrix}H\\H\end{matrix} + (C) \qquad (B) + (C){<}\begin{matrix}H\\H\end{matrix}$$

Such a scheme would be applicable to the higher plants in general inasmuch as no specific oxygenase is involved. If such a system were involved in the respiratory mechanism either

$$(A){<}\begin{matrix}H\\H\end{matrix} \quad \text{or} \quad (B){<}\begin{matrix}H\\H\end{matrix}$$

might represent the respiratory substrate, but Harrison does not explain which of them is to be so regarded.

It will be observed that Keilin's theory of the oxidation process in respiration is concerned with seeking an explanation of the mechanism of oxidation in living cells without specific reference to the nature of the substances oxidized or the substances resulting from oxidation. It is concerned in particular with the relation of cytochrome to the oxidizing enzymes of the cell. It offers no definite suggestion of the actual stages in the breaking down of more complex substances into carbon dioxide and water, nor does there appear to be any direct evidence that oxidation by means of the cytochrome system involves the production of carbon dioxide.

A more complete theory of respiration was put forward by Palladin in 1912. Palladin was of opinion that all the carbon dioxide produced in respiration is formed during the anaerobic stage of the process which involves the dissociation of water, the oxygen of which oxidizes the respiratory substrate. The hydrogen may reduce intermediate products of anaerobic respiration to give ethyl alcohol, or it may unite with some hydrogen acceptor as, for example, a respiration pigment. In this case the anaerobic stage of the respiratory process may be represented by the equation :

$$C_6H_{12}O_6 + 6H_2O + 12R = 6CO_2 + 12RH_2$$

This process can only continue until the hydrogen acceptors have taken up their maximum quantity of hydrogen ; the reaction must then cease and no more carbon dioxide can be formed under anaerobic conditions.

But if oxygen is present the reduced hydrogen acceptors (respiration chromogens) become oxidized owing to removal of hydrogen to form water, this reaction taking place by the action of peroxidase. The anaerobic reaction (or chain of reactions) is then able to proceed owing to the regeneration of hydrogen acceptors (respiration pigments). The aerobic stage of respiration is thus not concerned with the production of carbon dioxide but with the regeneration of the hydrogen acceptor which enables the anaerobic production of carbon dioxide to proceed. This stage in the process may be represented by the equation :

$$12RH_2 + 6O_2 = 12R + 12H_2O$$

The two end products of aerobic respiration, carbon dioxide and water, are thus produced in different stages of the process.

Palladin's view of the mechanism of the respiration process is decidedly hypothetical and rests

largely on the behaviour of respiration pigments and chromogens. It is not certain that these latter are universally present in plants, although when absent other hydrogen acceptors and donators might play the same part. A more serious criticism of the theory is that it does not fall readily into line with Neuberg's theory of yeast fermentation, which rests on a fairly solid foundation of observed facts. But wh le we may regard Palladin's theory as mainly of historical interest, it cannot be regarded as definitely disproved.

An analysis of data on respiration of apples led F. F. Blackman in 1928 to put forward a general scheme of the respiratory process, which is in harmony both with Neuberg's theory of fermentation and with the conceptions of Meyerhof and his pupil Genevois on the processes of aerobic respiration, based on work with muscle, yeast and sweet peas.

Beginning with those substances in the apple which serve as reserve carbohydrates, Blackman's scheme involves a chain of four consecutive processes ; (1) hydrolysis, (2) activation, (3) glycolysis, and (4) 'respiration in a restricted sense'.

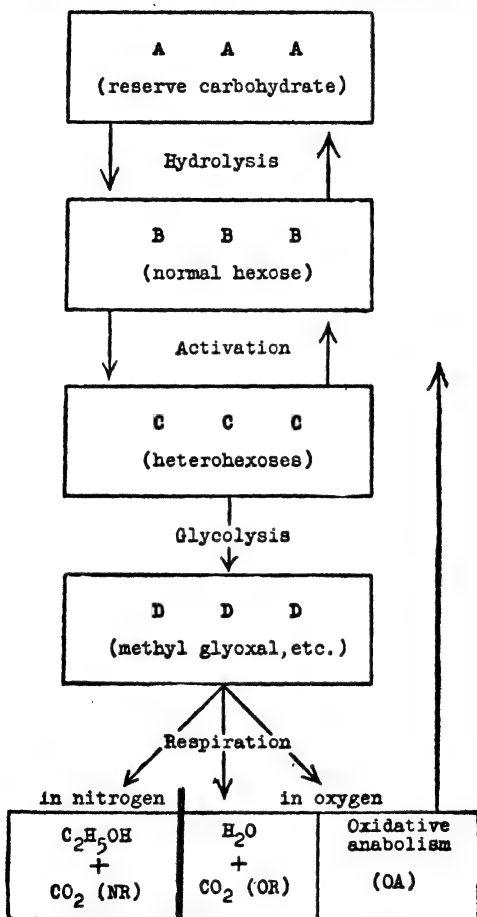
In the first process reserve carbohydrates such as starch and cane sugar are hydrolysed to free normal hexoses. These are not respired directly but undergo 'activation'. Blackman describes activation as 'leading to the formation of hexoses of the group of heterohexoses with the less stable type of internal ring structure'. It thus consists of an isomeric change of the hexose molecule and appears to be nothing other than the formation of γ-sugars for which we have already seen there is considerable evidence.

The next stage, glycolysis, is brought about by enzymes of the zymase complex. We have seen that in absence of oxygen a number of intermediate substances of anaerobic respiration are probably produced, such as methyl glyoxal, pyruvic acid,

acetaldehyde and possibly lactic acid. In Blackman's scheme, by the glycolysis stage is meant the breaking down of activated hexose to these various substances.

The fourth stage, respiration in the special narrow sense, is the production of the final product from these intermediate substances. Up to the formation of these substances the course of respiration is qualitatively the same under aerobic and anaerobic conditions, for zymase action is independent of the presence or absence of oxygen. The last stage, on the other hand, is quite different according as conditions are anaerobic or aerobic. Under anaerobic conditions in an atmosphere of nitrogen the products are alcohol and carbon dioxide. In presence of oxygen, on the other hand, the reactants may meet with one or other of two different fates. Part of the carbon is completely oxidized to water and carbon dioxide, but the carbon dioxide so formed only accounts for a fraction of the carbon which appears to be involved in glycolysis. The rest of the carbon must therefore have some other fate. No new substance appears to accumulate in the tissues, and the conclusion is therefore drawn that in presence of oxygen part of the products of glycolysis are worked back into the system, whether to the hexose or to some intermediate stage cannot be said. To this process the name 'oxidative anabolism' is given. Proof of such a synthesis of carbohydrate associated with oxidation forms an integral part of the already mentioned contribution by Meyerhof to our knowledge of this subject. Blackman's scheme may then be summarized as shown on page 103.

It is to be noted that the first two reactions are reversible but that glycolysis is irreversible.

It will thus be observed that the stages in the whole process are essentially those which have been hypothesized as the result of work of Neuberg, Meyerhof and others. We may therefore briefly

summarize Blackman's conclusions and the reasons which led him to them.

Let us first note the effect produced on respiration

of apples by changing the oxygen content of the environment. When air is replaced by pure oxygen the respiration rate increases, the rate in oxygen being 1·4 times the rate in air, while when normal air is replaced by air containing only 5 per cent.

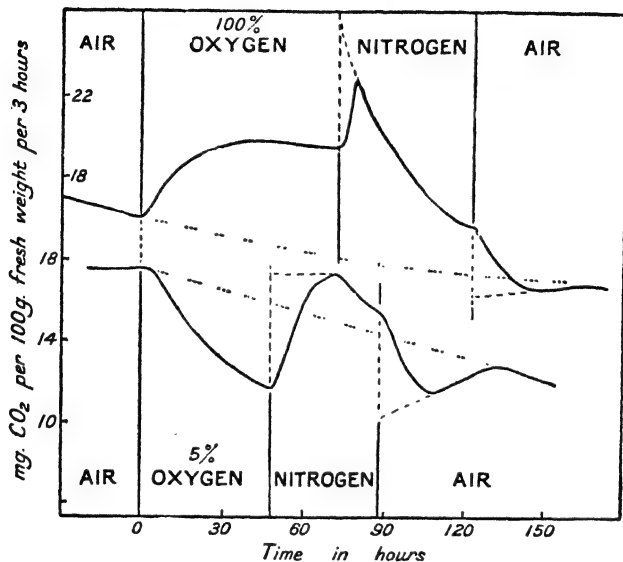



Fig. 10.—Curves showing the effect on carbon dioxide output of Bramley's Seedling apples, produced by atmospheres containing various concentrations of oxygen (*After* Blackman)

oxygen the respiration rate falls to 0·7 that in normal air, or less. The transition to the new rate is, however, slow in both cases, lasting at least 45 hours (cf. Fig. 10). Now it seems most unlikely that it would take so long a time for a pure oxidation rate to adjust itself after an increase in oxygen concentration, and it is therefore concluded that oxygen

concentration must influence the rate of some earlier stage in the process. It is known that zymase action is independent of oxygen, so it is presumed that oxygen concentration must affect a pre-glycolytic stage, with the result that increase in oxygen concentration brings about an increase in the concentration of the effective substrate for glycolysis. As this increase in the concentration of the substrate is maintained it means that increase in oxygen concentration results in an increase in the rate of production of the substrate, that is, the heterohexoses, from the normal hexoses, and in consequence an increase in the rate of production of these from the reserve carbohydrates.

That oxygen concentration influences the rate of production of the effective substrate for glycolysis from normal hexoses and reserve carbohydrates is, moreover, suggested by the slow rate of adjustment which is characteristic of altered carbohydrate balance relations. The course of respiration in the transition 'is exactly that of an approach to a reversible equilibrium. . . . We picture the opposed processes to be increased production of C from A — B by oxygen activation working against increased consumption of C by glycolysis, which rises with each rise of concentration until the two become adjusted to equality again.'

It will be further observed that these considerations suggest that the effective substrate in glycolysis is present in low concentrations since changes in oxygen concentration bring about marked changes in its production and consumption. For this reason a pre-glycolytic reaction consisting merely of the hydrolysis of reserve carbohydrates to normal hexoses is insufficient to meet the case since the normal hexoses are present in relatively high concentration and would therefore undergo slight alterations in concentration.

Such are the considerations which led Blackman

to postulate the heterohexoses as the direct substrate for glycolysis, and not normal hexoses. We have already noted that there is a considerable quantity of independent evidence in support of such a view.

Blackman's conclusions regarding the course of aerobic respiration subsequent to glycolysis are largely based on Parija's observations, to which reference has been made earlier, on respiration of apples in air and in nitrogen. It will be recalled that when air is replaced by nitrogen the initial rate of respiration in nitrogen at the moment of transference is 1·33 or 1·5 times the rate in air, but this rate slowly falls to a level which may be that of respiration in air, or somewhat higher. This slow fall is that slow transition which, as we have just seen, is characteristic of a change in oxygen concentration, and indicates the pre-glycolytic decrease in production of heterohexoses resulting from the fall in oxygen concentration from 20 per cent. to nothing. At the beginning, therefore, of the nitrogen period, the concentration of glycolytic substrate, which determines the rate of glycolysis, is that obtaining in air. In other words, the initial value of respiration in nitrogen (actually obtained by extrapolation to allow for the diffusion lag as explained in Chapter III) gives a value for glycolysis in air.

Now in anaerobic respiration only one-third of the carbon acted upon in the glycolytic stage finally appears as carbon dioxide; the other two-thirds appear as ethyl alcohol. If, then, the carbon atom is taken as a unit, we can say that respiration in nitrogen, as measured by carbon dioxide evolved, is only one-third of glycolysis, or using Blackman's nomenclature, Gl = 3NR, where Gl signifies glycolysis and NR nitrogen respiration.

Now we have seen that when air is replaced by nitrogen, before the rate of glycolysis has altered, the respiration increases to 1·5 or 1·33 times that in

air. Consequently, using the symbol OR for respiration in air, we have

NR = 1·5 OR (or 1·33 OR)

and Gl = 4·5 OR (or 4 OR)

This means that under aerobic conditions only one atom of carbon out of every 4 or 4·5 subjected to glycolysis is actually released as carbon dioxide. Consequently for every atom of carbon released as carbon dioxide 3 or 3·5 atoms of carbon are worked back into the system, or, again using Blackman's system of nomenclature, OA (oxidative anabolism) = 3·5 OR, (or 3·0 OR). It is not clear whether these different ratios (3·5 and 3·0) represent the extremes of a range, or whether the ratios remain constant within a given type. The available evidence appears to point to the latter alternative. If this is so, the lower ratio appears to be associated with late metabolic states of the apple and the higher ratio with earlier ones.

The question naturally arises as to what is the fate of the carbon subjected to oxidative anabolism, and it may be said at once that there is at present no answer to the question. This carbon may be built back to the hexose stage or to some other substance such as malic acid. But if any such substance is formed, it must be consumed as rapidly as it is produced, for there is no evidence of any such product accumulating.

It is to be observed that although the products of oxidative anabolism result from an oxidation process it does not follow that they are themselves oxidized derivatives of the products of glycolysis. They might be reduced derivatives formed along with the oxidized product, carbon dioxide, in which case the last stage in aerobic respiration would be an oxido-reductive process catalysed by an oxidoreductase.

It should be noted that in Blackman's scheme

oxygen plays a double part in respiration. Not only does it bring about the oxidation of the products of glycolysis, but it also affects the pre-glycolytic stages in that it brings about an alteration in the rate of production of the direct substrate for glycolysis.

Yet one further point should be mentioned. This scheme as it at present stands has so far been shown to be applicable only to senescent apples and possibly to other ripe fruits. Recent work suggests that in the case of other tissues only parts of the scheme can be applicable. The hypothesis, however, has marked a stage in the development of the theory of respiration and has acted as a strong stimulus to research into this fundamental property of life.

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